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EFFICACY OF DIFFERENT STRAINS OF BRUCELLA ABORTUS AS IMMUNIZING AGENTS AGAINST INFECTIOUS ABORTION[1]

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PURPOSE OF THE INVESTIGATION

Previous experiments[2] with a strain of bovine *Brucella abortus*,[3] which through long cultivation on artificial media had become so nearly avirulent for guinea pigs that it would no longer cause lesions in them except when injected in very large doses, showed that it possessed considerable value in protecting these animals from developing lesions of abortion disease when they were subsequently injected with a virulent strain of *Br. abortus*. Huddleson[4] showed that a similar avirulent culture not only increased the resistance of guinea pigs to *Br. abortus* but also seemed to induce some degree of immunity in cattle.

Numerous attempts made at the Experiment Station, Bethesda, Md., about 10 years ago, to infect cattle by natural means with the swine type of *Brucella abortus* failed. These failures suggested the possibility that this type might be used to advantage in preparing an effective vaccine that would be free from the danger of infecting the treated animals. This idea was evolved before the greater danger of the swine type to human health was generally realized. It was hoped that both the avirulent bovine strain and the swine strain could be used on pregnant as well as on nonpregnant cattle without infecting them. Arrangements therefore were made to try these two strains on cows and heifers both before and after breeding; and, for the sake of comparison, to include a group of animals vaccinated, before service, with a virulent bovine strain.

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[1] Received for publication March 10, 1932; issued December, 1932. This investigation was begun under the direction of E. C. Schroeder, former superintendent of the Experiment Station, to whom credit is given for a large share of the planning of the experiments and help in carrying them out. His untimely death in January, 1928, occurred before the work was completed.

[2] SCHROEDER, E. C., and COTTON, W. E. RECENT BUREAU OF ANIMAL INDUSTRY EXPERIMENT STATION BOVINE INFECTIOUS ABORTION STUDIES. Jour. Amer. Vet. Med. Assoc. 66: 550-561. 1925.

[3] This organism was formerly referred to as *Bacillus abortus* or *Bacterium abortus*.

[4] HUDDLESON, I. F. STUDIES ON A NONVIRULENT LIVING CULTURE OF BACT. ABORTUS TOWARDS PROTECTIVE VACCINATION OF CATTLE AGAINST BOVINE INFECTIOUS ABORTION (BANG'S ABORTION DISEASE). Mich. Agr. Expt. Sta. Tech. Bul. 65, 36 p. 1924.

——— THE VACCINAL IMMUNIZATION OF ANIMALS AGAINST BACT. ABORTUS (BANG) INFECTION. U. S. Livestock Sanit. Assoc. Proc. (1925) 29: 210-215. 1926.

SOURCES OF VACCINES

The avirulent bovine strain used in these investigations was isolated from the milk of a cow in 1915 and was then virulent. For many months this strain lived on culture media in a single tube so completely sealed with paraffin that there was practically no loss of moisture. After this period it was kept alive by frequent transfer on culture media without resort to animal passage. At the time of the experiment it had been avirulent for guinea pigs for several years, so far as producing lesions was concerned, but the microorganisms could be recovered from the spleens of the guinea pigs, in some instances, two months after inoculation. Though of very low virulence, the strain still retained its agglutinability in full measure. Four and one-half years before the experiment was begun, a pregnant heifer, negative to the blood test for abortion, was injected intravenously with the growth from one agar slant of this strain suspended in 20 c c of physiological salt solution. Thirty-seven days later she aborted an 8-month fetus and reacted to the agglutination test for abortion with a titer of 1 to 1,600. Guinea pigs inoculated with material from her placenta developed characteristic lesions of abortion disease, but those inoculated with her colostrum failed to do so. The cow's blood, a year later, was negative to the agglutination test. Nine months after the injection of the heifer mentioned above, two pregnant cows given three feedings of the attenuated culture failed to become infected or to react to the agglutination test.

The swine strain used in the investigation was from a sow that had acquired abortion disease through natural exposure to infected swine. This strain had been kept alive in a guinea pig for a number of months. The organism was isolated from the guinea pig a few months before the beginning of the experiment and produced well-marked characteristic abortion-disease lesions of the swine type in guinea pigs.

The virulent bovine strain was isolated from an aborting cow about six months before it was used in the experiment. It produced well-marked abortion-disease lesions of the bovine type in guinea pigs.

TESTS OF THE THREE VACCINES WHEN USED BEFORE CONCEPTION

METHOD AND TIME OF VACCINATION

To test the efficacy of the three vaccines when used before conception, they were administered to three groups of cows and heifers negative to the agglutination test for infectious abortion, and a similar group, untreated, was reserved for control. Each of the vaccines was prepared from four days' growth on 11 agar slants suspended in approximately 140 c c of 0.85 per cent salt solution, and all were of about the same density.

Each of the cattle in the first three groups received subcutaneously 20 c c of vaccine, 10 c c in front of each shoulder. With the exception of cow 1155 in the second group, the animals were vaccinated December 8, 1926. There was little systemic reaction in the cattle after vaccination, and the reactions were not very marked nor did they persist for long.

TABLE 1.—*Dates of vaccination and breeding of experimental cattle vaccinated before conception*

GROUP 1.—VACCINATED WITH AVIRULENT BOVINE STRAIN

Animal No.	Date of vaccination	Date of breeding	Interval between vaccination and breeding
Cow:	1926	1927	*Days*
1227	Dec. 8	Apr. 8	121
1365	do	Mar. 17	99
Heifer:			
1341	do	May 18	161
1344	do	Apr. 19	132
1348	do	Apr. 8	121
1369	do	Mar. 29	111

GROUP 2.—VACCINATED WITH VIRULENT SWINE STRAIN

Cow:			
1209	Dec. 8	Mar. 18	100
1155 [a]	Dec. 13	Apr. 5	113
1370	Dec. 8	do	118
Heifer:			
1346	do	Mar. 28	110
1350	do	Mar. 16	98
1356	do	Apr. 19	132

GROUP 3.—VACCINATED WITH VIRULENT BOVINE STRAIN

Cow:			
1142	Dec. 8	Apr. 5	118
1362	do	Mar. 22	104
1366	do	Apr. 5	118
Heifer:			
1345	do	Mar. 28	110
1347	do	May 16	159

GROUP 4.—CONTROLS, NOT VACCINATED

Cow:			
1248		July 8	
1340		May 14	
Heifer:			
1322		Apr. 2	
1323		do	
1325		May 11	
1342		Apr. 15	
1355		Mar. 17	

[a] Cow 1155, which had been vaccinated on Dec. 13, 1926, too late in pregnancy to be used in another experiment for which she was intended, was allowed to produce her calf, again bred Apr. 5, 1927, and added to this group to replace an animal that proved to be pregnant.

The cattle were served on the dates given in Table 1. With the exception of control cow 1248, all the animals in the experiment conceived within a period of 63 days of one another.

To test the virulence of the three vaccines, each was injected subcutaneously into 12 guinea pigs, the doses varying from $\frac{1}{64}$ to $\frac{1}{4}$ c c for the swine strain and virulent bovine strain, and from $\frac{1}{4}$ to 2 c c for the avirulent bovine strain. All guinea pigs receiving the swine and virulent bovine strains developed lesions of abortion disease characteristic of the respective types. None of the guinea pigs that received the avirulent bovine strain developed lesions.

EXPOSURE OF ANIMALS TO BRUCELLA ABORTUS

The four groups of animals were given six exposures to *Brucella abortus* infection by ingestion at intervals of from 7 to 17 days within a period of 55 days. The first of these exposures was made August 2, 1927, at which time most of the animals were from three and one-half to four and one-half months pregnant. In addition to the ingestion exposures, two drops of the material used in making them were instilled into one eye of each animal on two of the dates, August 29 and September 9, on which these exposures were made.

It was intended to use the organs of aborted bovine fetuses for the exposures, but only one such fetus could be obtained for the last exposure. For the remaining five exposures, therefore, use was made of diseased organs of guinea pigs that had been inoculated with fetal and placental material from aborting cows and of recently isolated *Br. abortus* cultures. Cultures alone were used in making two of the exposures, and cultures and guinea-pig tissues in the remaining ones. Emulsions were made of the bovine fetal material and of the guinea-pig organs by grinding in a mortar. Enough 0.85 per cent salt solution was added to make the mass sufficiently fluid to pass easily through a syringe, after which it was strained through cheesecloth to remove coarser particles. The suspensions of *Br. abortus* were prepared by washing the growth from 6-day agar slants with 0.85 per cent salt solution.

The exposures were made by placing the infectious material on the root of the tongue by means of a syringe. When the tissue emulsions were given, the dose was 40 c c. When the culture was given, the dose was equivalent to the growth on one agar slant suspended in 40 c c of 0.85 per cent salt solution. When tissue emulsions and cultures were combined, *Br. abortus* was added at the rate of about half the growth on one agar slant per animal, the dose of the combined materials being 40 c c.

Six guinea pigs were injected with each lot of material to test its virulence. These animals, in all cases, showed marked lesions of abortion disease when they were autopsied two months later. Had there not been doubt, at the time the exposures were made, of the virulence of the material used, fewer exposures would have been given.

The results of the exposures of the animals in the different groups vaccinated before conception follow. Tests of placentae, uterine discharges, fetal organs, colostrum, and milk were made for *Br. abortus* by guinea-pig inoculations unless otherwise noted.

GROUP 1. CATTLE VACCINATED WITH AVIRULENT BOVINE STRAIN

Cow 1227. Aborted September 26, 1927, 55 days after first exposure and 171 days after service, or in the sixth month of pregnancy; *Br. abortus* isolated from fetal organs by cultural methods; placenta and fetal organs produced typical abortion-disease lesions in guinea pigs; milk on November 23, 1927, and milk and supramammary glands on March 30, 1928, the date on which cow was killed, negative for *Br. abortus*.

Cow 1365. Gave birth to premature calf, which lived about an hour, October 11, 1927, 208 days after service and 70 days after first exposure; uterine discharges infected; milk positive for *Br. abortus* October 22, but negative November 22, 1927, and January 11, 1928; organs of calf negative for *Br. abortus*.

Heifer 1341. Produced a full-term (287-day) calf February 29, 1928, 211 days after first exposure; afterbirth normal in appearance and negative for *Br. abortus;* colostrum found to be infected March 1.

Heifer 1344. Gave birth to calf January 17, 1928, 273 days after service and 168 days after first exposure; calf died, without taking nourishment, a few hours

after it was born; afterbirth of normal appearance, passed normally, and negative for *Br. abortus;* stomach fluid and organs of calf negative. Though the record of this cow is incomplete because no tests were made of her milk for *Br. abortus*, agglutination tests of her blood, shown in Table 3, strongly indicate that she did not become infected. The calf, though slightly premature, probably died because of exposure to cold.

Heifer 1348. Gave birth to calf December 11, 1927, 247 days after service and 131 days after first exposure; calf died without taking nourishment; portion of afterbirth passed the next day had characteristic abortion-disease lesions; both it and organs of calf positive for *Br. abortus;* milk infected on 19 occasions and not infected on 2 others, at intervals from February 4 to November 28, 1928.

Heifer 1369. Gave birth to apparently normal calf December 27, 1927, 273 days after service and 147 days after first exposure; afterbirth much diseased and infected with *Br. abortus;* milk negative on February 25 and 28, 1928, but slightly infected March 3.

Group 2. Cattle Vaccinated with Virulent Swine Strain

Cow 1209. Aborted November 18, 1927, 245 days after service and 108 days after first exposure; placenta, fetal organs, and colostrum infected with *Br. abortus;* milk infected January 11 and 28 and February 4, 25, and 28, 1928.

Cow 1155. It was intended to use this animal in the experiment in vaccination after conception, but she was vaccinated so late in pregnancy that she produced a calf January 5, 1927, before she was exposed. She therefore received somewhat different treatment than the remaining animals in the group, being vaccinated 23 days before parturition and 114 days, or nearly 4 months, before the next service. She produced apparently normal calf January 17, 1928, 287 days after service and 137 days after first exposure; afterbirth of normal appearance and negative for *Br. abortus;* milk also negative on eight different days from February 25 to September 19, 1928, inclusive.

Cow 1370. Gave birth to apparently normal calf January 12, 1928, 282 days after service and 163 days after first exposure; afterbirth of normal appearance and negative for *Br. abortus;* milk negative February 24 and 28 and March 3, 1928, but when cow was killed April 28, 1928, milk taken from her udder and an emulsion of her supramammary glands produced abortion-disease lesions of the swine type in guinea pigs.

Heifer 1346. Gave birth to apparently normal calf December 29, 1927, 276 days after service and 149 days after first exposure; afterbirth passed normally and lost; uterine discharges negative for *Br. abortus;* milk negative on seven different days from January 11 to April 28, 1928, as were also the supramammary glands when the cow was killed April 28, 1928.

Heifer 1350. Aborted October 19, 1927, 217 days after service and 78 days after first exposure; afterbirth retained; *Br. abortus* isolated from lungs, liver, spleen, and stomach fluid of fetus; afterbirth infected; milk negative for *Br. abortus* October 22, 1927, and January 11 and 28 and February 25, 1928.

Heifer 1356. Aborted November 1, 1927, 196 days after service and 91 days after first exposure; fetal organs and uterine discharges infected with *Br. abortus*, but milk negative for this microorganism February 25 and 28 and March 3, 1928.

Group 3. Cattle Vaccinated with Virulent Bovine Strain

Cow 1142. Gave birth to apparently normal calf January 11, 1928, 281 days after service and 162 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus*, but milk found to be infected with this microorganism January 28, February 25 and 28, and March 3, 1928.

Cow 1362. Gave birth to apparently normal calf December 30, 1927, 283 days after service and 150 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus;* milk also negative January 28, February 4 and 28, and March 3, 1928, but produced slight abortion-disease lesions in one of two guinea pigs inoculated February 25, 1928.

Cow 1366. Died of enteritis October 7, 1927, 66 days after first exposure; uterus contained a 6-month fetus; uterus and contents apparently normal; guinea pigs inoculated from fetal organs, placenta, udder, and supramammary glands all remained normal.

Heifer 1345. Gave birth to apparently normal calf December 31, 1927, 278 days after service and 151 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus;* milk also negative January 11, February 28, March 3, and October 12, 1928.

Heifer 1347. Gave birth to apparently normal calf February 15, 1928, 275 days after service and 197 days after first exposure; afterbirth passed normally,

of normal appearance, and negative for *Br. abortus;* milk negative February 24 and March 3, 1928.

Cattle 1142, 1362, 1345, and 1347 were again served within the first and second quarters of 1928 and were exposed during the periods of gestation which followed, along with cattle in another experiment and their controls. The exposures were made on August 3 and 9, when the animals were in their fifth, fifth, third, and fourth months of gestation, respectively. The exposure was sufficiently severe to cause two of five controls to become infected, one of which aborted and the other gave birth to a weak calf on the two hundred and fifty-eighth day of her gestation period. All four of the vaccinated animals in group 3, though they had received no protective treatment after vaccination and exposure, produced apparently normal, full-term calves. Guinea pigs inoculated with portions of placenta and uterine discharges from three of the cows were negative for *Br. abortus* on autopsy. No inoculations were made from the placenta of cow 1345, since her blood was negative to the agglutination test and had been so for more than six months. The colostrum of cow 1142 contained *Br. abortus*, but that from cows 1347 and 1362 did not, as shown by guinea-pig inoculations. The infection of the udder of cow 1142 dated from either the exposure or vaccination of the previous year, probably the latter, since her blood reaction persisted in a titer of 1 to 800 or higher after vaccination.

Group 4. Control Cattle

Cow 1248. Aborted February 23, 1928, 230 days after service and 205 days after first exposure; afterbirth had characteristic lesions of abortion disease; stomach and intestinal fluids and organs of fetus and placenta infected with *Br. abortus;* milk infected February 24 and 28 and March 30; cow was killed March 30 and her supramammary glands found to be infected.

Cow 1340. Gave birth to apparently normal calf February 23, 1928, 285 days after service and 205 days after first exposure; afterbirth lost; uterine discharges all negative for *Br. abortus* as to lesions and agglutination test; milk negative February 28 and March 3.

Heifer 1322. Aborted December 4, 1927, 246 days after service and 124 days after first exposure; afterbirth showed typical lesions of abortion disease; innumerable colonies of *Br. abortus* isolated from lungs of fetus; other organs and stomach and intestinal fluids of fetus and afterbirth infected with *Br. abortus* as was also milk January 11 and 28, February 4, 25, and 28, and March 3, 1928.

Heifer 1323. Aborted December 9, 1927, 251 days after service and 129 days after first exposure; afterbirth showed characteristic abortion-disease lesions; afterbirth infected with *Br. abortus* as were also organs and stomach and intestinal contents of fetus; no milk tests were made.

Heifer 1325. Aborted November 10, 1927, 183 days after service and 100 days after first exposure; afterbirth lost but uterine discharges characteristic of *Br. abortus* infection, and this microorganism isolated by cultural methods from stomach fluid, spleen, and lungs of fetus; uterine discharges infected but milk not infected November 22, 1927, the only time that it was tested.

Heifer 1342. Aborted November 12, 1927, 211 days after service and 102 days after first exposure; organs and stomach fluid of fetus and placenta and colostrum infected with *Br. abortus;* milk also infected November 22, 1927, February 25 and 28, March 3, August 11 and 29, October 20, 22, 23, 25, and 31, November 2, 7, 10, 15, and 20, 1928, producing marked lesions in all guinea pigs inoculated.

Heifer 1355. Gave birth to apparently normal calf December 19, 1927, 277 days after service and 139 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus;* milk slightly infected February 25 but negative January 11 and 28, February 28, March 3, and April 12, 1928.

The results obtained in the tests with cows and heifers vaccinated before conception are presented in condensed form in Table 2. This

summary of data permits a ready comparison of the four groups. In the last two columns showing the relative resistance and percentage of full-term calves which lived, the reader will observe that both the percentage of resistance to infection and the percentage of living calves are much higher in the cattle vaccinated with the virulent bovine strain than in those vaccinated with the virulent swine or the avirulent bovine strains, and very much higher than in the controls. Furthermore, the percentage of udder infection is less in the cattle vaccinated with the virulent bovine and swine strains than in the controls. The significance of the data, however, is obviously somewhat limited because of the small number of cattle in the groups.

TABLE 2.—*Comparative resistance to Brucella abortus exposure of cows and heifers vaccinated with different strains of Br. abortus before breeding and those not vaccinated*

Group No.	Cattle	Strain of vaccine	Abortions or premature calves		Cows producing full-term calves		Cows producing infected or noninfected milk		Cows entirely resisting infection	Full-term calves which lived
			Caused by Br. abortus	Not caused by Br. abortus	Afterbirth infected	Afterbirth not infected	Infected	Noninfected		
	Number		*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*
1	6	Avirulent bovine	3	1	1	1	3	[a] 2	[b] 33.3	33.3
2	6	Virulent swine	3	0	0	3	[c] 2	4	33.3	50.0
3	[d] 4	Virulent bovine	0	0	0	4	2	2	50.0	100.0
4	7	None (control)	5	0	0	[e] 2	[f] 4	[f] 2	14.3	28.6

[a] No test was made of the milk of one of these animals, No. 1344, but agglutination tests of her blood and all other tests regarding her indicate that she entirely escaped infection.
[b] No. 1344 considered as being negative.
[c] Udder infection of 1 animal caused by vaccination and not by exposure.
[d] Originally 5 animals; 1 died, leaving 4 in the group as sources of data.
[e] Afterbirth of 1 animal lost but uterine discharges were negative for *Brucella abortus*
[f] Milk of 1 aborting animal was not tested.

EFFECTS OF VACCINATION AND EXPOSURE ON AGGLUTININ CONTENT OF BLOOD

Tables 3 to 6 give the results of agglutination tests made at intervals from the time of vaccination until after the termination of pregnancies. In general the tests were made in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, and 1:800. In a few instances the dilution was carried to 1:1,600 and 1:3,200. In some cases, when it seemed certain that the 1:25 dilution would not furnish additional data, it was omitted as were also the higher dilutions after the data obtained were deemed adequate.

The vaccine prepared from the avirulent bovine strain of *Br. abortus* (Table 3) induced a well-marked agglutination titer in the blood in 8 days. The titer was at its maximum at that time and had either entirely subsided or reached a low point 45 days later, except in one animal, in which it subsided more gradually.

The swine-strain vaccine (Table 4) induced the maximum agglutination titer in about 12 days and likewise soon began to subside, but did not so fully disappear as did the avirulent vaccine. The animal (No. 1370) in which the vaccine infected the udder retained a fairly high titer throughout the experiment.

The maximum titer induced by the virulent bovine-strain vaccine (Table 5) was reached by the sixteenth day and in two animals showed no tendency to subside, indicating that the vaccine had invaded the udder.

TABLE 3.—*Results of agglutination tests of blood sera of cattle in group 1 (vaccinated with an avirulent bovine strain of Brucella abortus before conception) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively* [a]

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1227	Cow 1365	Heifer 1341	Heifer 1344	Heifer 1348	Heifer 1369
	1926						
	Nov. 22	----------	-----	-----	-----	-----	-----
	Dec. 8	----------	-----	ss----	-----	-----	-----
	Dec. 11	-----	s----	s----	-----	-----	-----
	Dec. 16	++++++s	+++++s	++++p	+++++s	+++++p	+++p-
	Dec. 20	o++++++s	+++++s	o+++ss-	o++++s-	o+++++s	++ps--
	1927						
	Jan. 4	o++++++-	o++++p-	o++s---	o+++s--	o++++--	os------
Dec. 8, 1926, vaccinated	Jan. 22	o++ps---	o+ps----	o+s----	ops----	o+s----	o-------
	Feb. 5	o++s----	ops-----	oss-----	os------	os------	o-------
	Feb. 17	o+s-----	ops-----	os------	o-------	o-------	o-------
	Feb. 28	o+s-----	ops-----	os------	o-------	o-------	o-------
	Mar. 14	o+s-----	ops-----	os------	o-------	o-------	o-------
	Apr. 2	+ps---	ps----	pp----	s-----	+s----	s-----
	May 10	pss---	ps----	s-----	------	ps----	s-----
	July 11	+p----	ps----	s-----	s-----	+s----	s-----
	Aug. 3	ps----	ps----	s-----	------	+s----	s-----
	Aug. 9	+ps---	+s----	ps----	+s----	ps----	s-----
	Aug. 18	+ps---	+ps---	s-----	+p---·	++s---	+ps---
	Aug. 30	+ps---	++++++	s-----	+p----	ps----	pp----
	Sept. 13	++ps--	o+++++p-	ps----	+ps---	+++p-	++s---
	Sept. 23	o+++s----	o+++++-	++s---	ps----	o+++p-	++++-
	Sept. 26	[b]					
	Oct. 5		[c] o+++++ps	++p---	pps---	o+++p--	+++++s
Six exposures from Aug. 2 to Sept. 26, 1927, inclusive	Oct. 12	o++++++-	o+++++p-	+++p--	ps----	o+++p-	o++p---
	Nov. 3	o+++++++s	o+++++++-	o+++++s	+p----	o+++++	o+++s---
	Dec. 5		o++++++s-	o+++++p-	op-----	o++++++	o+++p---
	Dec. 12					[d] opp++++p	
	Dec. 23		o+++++p---	o++++++-	+s----	o+++++++p	[e] o+++p---
	1928						
	Jan. 11	o++++++s	o+++++s-	o+++++p	[f] p----	o+++++++p	o+p----
	Mar. 1			[g] o++++++p			
	Mar. 3				s-----		

[a] Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; -=no agglutination; o=no test made in this dilution.
[b] Sept. 26, aborted. [c] Oct. 16, aborted. [d] Dec. 11, calved prematurely. [e] Dec. 23, calved. [f] Jan. 17, calved prematurely. [g] Feb. 29, calved.

TABLE 4.—*Results of agglutination tests of blood sera of cattle in group 2 (vaccinated with virulent swine strain of Brucella abortus before conception) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively* [a]

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1209	Cow 1155 [b]	Cow 1370	Heifer 1346	Heifer 1350	Heifer 1356
	1926						
	Nov. 22	-----	-----	-----	-----	-----	-----
	Dec. 8	-----		-----	-----	-----	s----
	Dec. 11	-----		-----	s----	p----	-----
	Dec. 13		-----				
	Dec. 16	+++++++s	-----	+++ps	+++p-	+++++	++++p
	Dec. 20	o++++++	o++ps--	o++++s-	o+++++p	o+++++s-	o++++p
	1927						
Dec. 8, 1926, vaccinated	Jan. 4	o+++++s	[c]o++++ps	o++++s-	o+++++-	o+++++s	o++++s
	Jan. 22	o++++s-	o+++s-	o++++p--	o+++s--	o+++s--	o++s--
	Feb. 5	o+++ps-	o+ps-	o++++p-	o+++p---	o++s---	o+p----
	Feb. 17	o+++p---	o++s---	o++++p-	o+++----	o++s---	ops---
	Feb. 28	o+++ps--	o++s---	o+++++s	o+ps----	o++s---	o+s----
	Mar. 14	o+++s---	o+ps---	o+p++p-	opp----	o+ps---	op------
	Apr. 2	++ps--	o+ps--	o++++p-	o+++ps--	++s--	ps---
	May 10	++ps--	++ps-	o++++p-	++s--	++s--	ps---
	July 11	++p--		o+++p--	++s--	+ss--	s----
	Aug. 3	++ps-	ps---	+++ps-	+ps--	+p---	s----
	Aug. 9	+++---	++s--	++++p-	+p---	+p---	ps---
	Aug. 18	++ps--	+ps--	++++ps	++s--	++s--	+s---
	Aug. 30	++ps-	+pp--	++++ps	++s--	+ps--	++p--
	Sept. 13	++++s	++s--	o+++s--	+++--	+p---	+++s-
	Sept. 23	o+++ps	++s--	o+++p--	o+s---	+ps---	+++---
Six exposures from Aug. 2, to Sept. 26, 1927, inclusive	Oct. 5	o+++ps-	++s--	o++++s-	ops---	++p--	+++---
	Oct. 12	o+++---	+ps--	o+++s--	os------	[d]o+++s--	++p--
	Nov. 3	[e]o+++ps-	+ps--	o+++ps-	+p---	o+++++p	[f]o++s--
	Dec. 5	o+++++++	o+s---	o++ppp	[g]os----	o++++ps-	o+++s-
	Dec. 23	o++++++++	o+s---	o++++p-	ss---	o+++++-	o+++--
	Dec. 30				ps---		
	1928						
	Jan. 11	o+++++++	o+s---	[h]o++pps-	+s---	o++p--	o++s--
	Jan. 17		[i]++ps-	o++ppp			

[a] Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; -=no agglutination; o=no test made in this dilution.
[b] Cow 1155 vaccinated Dec. 13. [c] Jan. 5, calved. [d] Oct. 19, aborted. [e] Nov. 18, aborted. [f] Nov. 1, aborted. [g] Dec. 29, calved. [h] Jan. 12, calved. [i] Jan. 17, calved.

TABLE 5.—*Results of agglutination tests of blood sera of cattle in group 3 (vaccinated with virulent bovine strain of Brucella abortus before conception) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively* [a]

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1142	Cow 1362	Cow 1366	Heifer 1345	Heifer 1347
	1926					
	Nov. 22	−−−−−	−−−−−	s−−−−	−−−−−−	−−−−−
	Dec. 8	−−−−−	s−−−−	s−−−−	−−−−−	−−−−−
	Dec. 11	s−−−−	s−−−−	s−−−−	−−−−−	s−−−−
	Dec. 16	++++++s−	+++++s−	p++ps	++++++s	++++++s
	Dec. 20	o+++++p	o+++++s	o+++++s	++++++s−	o+++++p−
	1927					
Dec. 8, 1926, vaccinated	Jan. 4	o+++++p	o+++++p	o++++p−	o++ps−−	o++++p−
	Jan. 22	o+++++ps	o++++p−	o++s−−−	o+++++s	o+++−−−−
	Feb. 5	o+++++p	o++++−−	o++s−−−	o+p−−−−	o++s−−−
	Feb. 17	o+++++p	o+++p−−	o+++s−−	ops−−−−	o++−−−−
	Feb. 28	o++++++	o++++s−	o+++−−−−	ops−−−−	o++s−−−
	Mar. 14	o++++++s	o+++s−−	o++ps−−	os−−−−−	o++s−−−
	Apr. 2	o++++++s	o++ps	o++s−−−	+s−−−	++++s−−
	May 10	o++++++p	o++s−	+++s−	s−−−−	o++++s
	July 11	o++++++s−	++ps−	o+ps−−	s−−−−	o+++++
	Aug. 3	o+++++p−	+++s−	+++s−	s−−−−	o+++++p
	Aug. 9	o++++++s	+++s−−	+++p−−	s−−−−	o+++++p
	Aug. 18	o+++++ps	++ps−	+++ps .	ps−−−	o++++p−
	Aug. 30	o++++++p	++ps−	++ps−	ps−−−	o++++p−−
	Sept. 13	o++++++p−	++++s	++ps−	+s−−−	o++++ps−
	Sept. 23	o++++++s	o+++−−−	++s−−	ps−−−	o+++++−−
Six exposures from Aug. 2 to Sept. 26, 1927, inclusive	Oct. 5	o++++++s	o+++s−−	[b] ++ps−	ps−−−	o+++++p+−
	Oct. 12	o++++++s−	o++p−−	.....	ps−−−	o+++++++s
	Nov. 3	o++++++s	o+++s−	.....	os−−−−−	o+++++++
	Dec. 5	o++++++s	o++ps−−	.....	s−−−−	o+++++++p
	Dec. 23	o++++++p	[c] o++ps−	.....	[d] s−−−−	o+++++++−
	1928					
	[e] Jan. 11	o++++++−	o++p−−	.....	s−−−−	[f] o+++++p−

[a] Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; −=no agglutination; o=no test made in this dilution.
[b] Oct. 7, died. [c] Dec. 30, calved. [d] Dec. 31, calved. [e] Jan. 11, calved. [f] Feb. 15, calved.

TABLE 6.—*Results of agglutination tests of blood sera of cattle in group 4 (controls) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, 1:3,200, respectively* [a]

Date of first and last exposure	Date of agglutination test	Cow 1248	Cow 1340	Heifer 1322	Heifer 1323	Heifer 1325	Heifer 1342	Heifer 1355
Six exposures from Aug. 2, to Sept. 26, 1927, inclusive	1926 Nov. 22	------	------	------	------	------	------	------
	1927 Feb. 17	o------	o------	o------	-------	o-------	o------	o------
	Mar. 14	o------	o------	------	------	s----	s-	------
	July 11	s----	------	------	ss---	s----	s----	------
	Aug. 3	s----	------	------	ps---	s----	ps	------
	Aug. 9	+s---	s-----	s----	s----	s----	ps---	------
	Aug. 18	++s--	------	ps---	ps---	s----	pps--	s----
	Aug. 30	++ps-	p----	+ps--	+ps--	+ps--	++ps-	++++s
	Sept. 13	++p---	++p--	+ps--	+s---	+s---	+++++	o++++--
	Sept. 23	+ps---	p----	++s--	+p---	ps---	o++++p	o++++--
	Oct. 5	++s---	ps---	++----	+p---	++---	+++++	o+++--
	Oct. 12	++----	p----	+++++	+p---	o++++p	o++++++	o+++s--
	Nov. 3	++++p	ps---		+p---	o++++p		
	Nov. 7					[b] o++++p		
	Nov. 10							
	Nov. 12							
	Dec. 5	++ps-	------	[d] o++++++	o++ps		[c] o++++++++	
	Dec. 10				[e] o++++s-		o+++p+++	o+++p--
	Dec. 23	ops------	s----	o++++++p				(f)
	1928 Jan. 11	ps---	s----	o++++++-				o+++p-
	Feb. 24	[g] o++++p- -	[h] -----				o++++++	o+++p-
	Mar. 3			o+++++ps				

[a] Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; -=no agglutination; o=no test was made in this dilution.
[b] Nov. 10, aborted. [c] Nov. 12, aborted. [d] Dec. 4, aborted. [e] Dec. 9, aborted. [f] Dec. 19, calved. [g] Feb. 23, aborted. [h] Feb. 23, calved.

TESTS OF TWO OF THE THREE VACCINES WHEN USED AFTER CONCEPTION

METHOD AND TIME OF VACCINATION

A second series of experiments was made to test the efficacy of the vaccines on pregnant cows and heifers. The avirulent bovine strain and the virulent swine strain previously described were used, but the virulent bovine strain was omitted because of the obvious likelihood that it would cause abortion in pregnant animals. The vaccines were prepared in the same manner as before and were of about the same density.

The animals in groups 1 and 2 were vaccinated on December 13, 1926, except one animal in the latter group, which was vaccinated on December 8. Each animal in group 1 received 20 c c of the avirulent bovine-strain vaccine subcutaneously, divided between two points, one in front of each shoulder. The total dose for each animal represented the growth on two well-grown agar slants. Each animal in group 2 received the same quantity of the virulent swine-type vaccine in the same manner, the dose in this case being equivalent to the growth on one and one-third agar slants. Twelve control guinea pigs that were injected subcutaneously with doses varying from ¼ to 2 c c of the avirulent bovine strain showed no abortion-disease lesions when killed. Six control guinea pigs, similarly injected, that received doses varying from one-sixteenth to one-fourth cubic centimeter of the swine-strain vaccine, developed well-marked and typical swine-abortion-disease lesions.

The service dates of only two of the animals used in the experiment were available. The remaining animals were examined for pregnancy a few days before the principals were vaccinated, and were estimated to have been pregnant for the period shown in Table 7.

TABLE 7.—*Dates of vaccination and breeding of pregnant experimental cattle, 1926*

GROUP 1.—VACCINATED WITH AVIRULENT BOVINE STRAIN

Animal No.	Date of breeding[a]	Date of vaccination	Interval between vaccination and service[a]
Cow:			*Days*
1226	Sept. 14	Dec. 13	90
1361	Aug. 15	do	120
Heifer:			
1327	do	do	120
1367	July 16	do	150

GROUP 2.—VACCINATED WITH VIRULENT SWINE STRAIN

Cow: 1364	Aug. 30	Dec. 13	105
Heifer:			
1301	Sept. 14	do	90
1349	Aug. 15	do	120
1351	Sept. 14	Dec. 8	85
1358	Sept. 29	Dec. 13	75
1372[b]	May 1	do	226

[a] Dates and intervals between vaccination and service for cows 1174 and 1338 are exact, those for the remaining animals are approximate.

[b] This animal later was replaced by heifer 1351.

TABLE 7.—*Dates of vaccination and breeding of pregnant experimental cattle, 1926*—Continued

GROUP 3.—CONTROLS, NOT VACCINATED

Animal No.	Date of breeding	Date of vaccination	Interval between vaccination and service
			Days
Cow:			
1174	Sept. 10		
1338	Oct. 13		
Heifer:			
1300	Sept. 14		
1352	Aug. 30		
1357	Oct. 1		
1371	Sept. 8		

On December 14 and 15 some of the vaccinated cattle had elevated temperatures, but none were visibly sick. The local reactions were not very marked and subsided after a few days.

On January 5, 1927, cow 1155 of group 2 gave birth to an apparently normal calf; the afterbirth passed normally and was lost, but guinea pigs inoculated with uterine discharges remained normal. Milk on January 22, 1927, was negative for *Br. abortus*, by guinea-pig inoculations. On January 5, heifer 1343, of group 1, also gave birth to an apparently normal calf; the afterbirth was normal in appearance and negative for *Br. abortus*, by guinea-pig inoculations. Milk on January 22, 1927, also was negative for *Br. abortus*, by guinea-pig inoculations. Since both of these animals gave birth to calves before they were exposed, they were removed from this experiment.

EXPOSURE OF ANIMALS TO BRUCELLA ABORTUS

The animals in all three groups were given the same exposure, except that heifer 1351, which replaced heifer 1372, did not receive the first exposure, and heifers 1352 and 1371 were not given the last exposure, since their pregnancies terminated before it was made. The series of exposures extended over a period of 71 days.

In the first exposure, January 14, 1927, 5 c c of an emulsion of liver and stomach and intestinal contents of a bovine fetus, aborted as a result of natural infection, was placed on the root of the tongue of each animal in the three groups by means of a syringe with a piece of rubber tubing attached to its nozzle. *Brucella abortus* was isolated from the organs of the fetus, and 12 guinea pigs inoculated subcutaneously with from one-sixteenth to one-fourth cubic centimeter of the emulsion used in making the exposure developed typical bovine-abortion-disease lesions. The procedure was repeated on the following day, thus making the exposure of composite nature. Six guinea pigs inoculated with the emulsion on that day showed well-marked abortion-disease lesions at autopsy.

On February 5, 1927, heifer 1372 of group 2 gave birth to a normal calf; afterbirth was normal, and guinea pigs inoculated with it remained normal. As only 22 days had elapsed since exposure, this

heifer was replaced by heifer 1351. Heifer 1351 was vaccinated about five days earlier than the other animals in group 2, but otherwise received the same treatment.

In the second series of exposures, February 8, 9, and 10, 1927, each animal in the three groups was given, in the same manner as in the previous exposure, 10 c c of an emulsion of placenta of a cow that produced a premature calf which lived only about 24 hours. The placenta had the characteristic appearance of one infected with *Br. abortus*, and guinea pigs inoculated with the emulsion of it on each of the days it was given to the cattle developed abortion-disease lesions.

In the third series of exposures, February 23, 24, 25, 26, and 27, 1927, each animal of the three groups was given 20 c c of an emulsion of organs and stomach fluid of a bovine fetus, aborted as a result of natural exposure. Guinea pigs inoculated on each of these days with emulsion developed abortion-disease lesions.

In the fourth exposure, March 19, 1927, each animal in the three groups received 20 c c of an emulsion of organs and stomach fluid of a fetus from a cow that had been artificially infected to obtain material for exposure. In addition, two drops of the stomach fluid was placed in one eye of each of the cattle. Inoculation of guinea pigs proved the emulsion to be infectious but not markedly so.

In the fifth exposure, March 26, 1927, each animal in the three groups, except heifers 1352 and 1371 of group 3, whose pregnancies had terminated before the exposure was made, was given 20 c c of stomach fluid of the fetus from a cow that aborted on March 22, 1927. Guinea-pig inoculations showed that the material was thoroughly infectious.

The results of the exposures of the animals in the different groups vaccinated after conception follow. Tests of placentae, uterine discharges, fetal organs, colostrum, and milk were made for *Br. abortus* by guinea-pig inoculations unless otherwise noted.

Group 1. Cattle Vaccinated with Avirulent Bovine Strain

Cow 1226. Gave birth to normal calf June 27, 1927, 164 days after first exposure and 93 days after the last; afterbirth normal in appearance but infected with *Br. abortus;* milk, however, negative for this microorganism on July 11, 14, and 20 and October 6, 1927, and on January 12 and 28, February 25 and 28, and March 3, 1928.

Cow 1361. Aborted a small, 7½ to 8 months' fetus April 20, 1927, 96 days after first exposure and 25 days after the last; afterbirth not firmly retained but infected with *Br. abortus;* stomach fluid and organs of fetus not infected; milk not infected July 11, 14, and 20, and November 23, 1927, and January 12 and 28, and February 4, 1928; again became pregnant and on June 5, 1928, gave birth to apparently normal calf; afterbirth negative for *Br. abortus.*

Heifer 1327. Gave birth to apparently normal calf May 24, 1927, 130 days after first exposure and 59 days after the last; a few cotyledons of the placenta showed lesions of abortion disease and were found to be infected, as was also the milk July 11, 14, and 20, 1927. Cow was killed August 4, 1928; autopsy showed all organs to be apparently normal but supramammary glands proved to be infected with *Br. abortus.*

Heifer 1367. On April 5, 1927, udder developed, and at times from April 5 to 10 discharges from the vagina; on April 6 and 9 discharges used to inoculate guinea pigs but without infecting them. Gave birth to apparently normal calf April 25, 101 days after first exposure and 30 days after the last; afterbirth normal and proved to be negative for *Br. abortus;* milk negative for this microorganism July 11, 14, and 20 and November 23, 1927, and January 12 and 28, February 4, 25, and 28, and March 3, 1928. Gave birth to another apparently normal calf August 1, 1928; afterbirth negative for *Br. abortus.*

GROUP 2. CATTLE VACCINATED WITH VIRULENT SWINE STRAIN

Cow 1364. Gave birth to apparently normal calf June 10, 1927, 147 days after first exposure and 76 days after the last; afterbirth of normal appearance and 12 guinea pigs inoculated from it remained normal. Milk negative for *Br. abortus* July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, May 8, 11, and 16, June 22, 26, and 29, and September 19, 1928.

Heifer 1301. Gave birth to apparently normal calf June 29, 1927, 156 days after first exposure and 85 days after the last; afterbirth apparently normal but 4 of 10 guinea pigs inoculated from it became infected. Milk negative for *Br. abortus* July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, and May 8, 11, and 16, 1928.

Heifer 1349. Gave birth to apparently normal calf May 18, 1927, 124 days after first exposure and 53 days after the last; afterbirth appeared normal, and proved to be negative for *Br. abortus;* milk also negative July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, May 8 and 11, June 22, 26, and 29, and September 19, 1928.

Heifer 1351. Gave birth to apparently full-term calf June 24, 1927, 136 days after first exposure and 90 days after the last; afterbirth apparently normal except for what seemed to be very small necrotic areas at the borders of several of the cotyledons. Ten guinea pigs inoculated with fragments of these cotyledons failed to develop lesions of abortion disease. Milk negative for *Br. abortus* July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, May 8 and 11, June 22, 26, and 29, and September 19, 1928.

Heifer 1358. Gave birth to small but strong and apparently full-term calf May 5, 1927, 111 days after first exposure and 40 days after the last; afterbirth apparently normal and proved to be negative for *Br. abortus*. Milk on July 11 and 14, 1927, produced lesions of the kind caused by the swine type of *Br. abortus* in guinea pigs; on July 20, 1927, very marked swine-type lesions with orbital abscesses; on October 6, 1927, and January 12 and 28, and February 4, 1928, lesions of the swine type; and on January 7, 1931, more than four years after vaccination, milk still infected with the same type of *Br. abortus*. Calf was killed October 10, 1927; guinea pigs inoculated from portal and mesenteric and pharyngeal glands negative for *Br. abortus*.

Heifer 1372. Gave birth to apparently normal calf February 5, 1927, 22 days after first exposure and before the second was made; afterbirth normal in appearance and negative for *Br. abortus;* milk negative March 22 and 26, April 2 and 6, July 11, 14, and 20, 1927, and May 8, 11, and 16, June 22, 26, and 29, and September 5, 1928. Animal was killed September 5, 1928; supramammary glands negative for *Br. abortus*.

GROUP 3. CONTROL CATTLE

Cow 1174. Gave birth to apparently normal calf June 15, 1927, 152 days after first exposure and 81 days after the last; afterbirth retained but proved to be negative for *Br. abortus;* milk also negative July 11, 14, and 20 and November 22, 1927.

Cow 1338. Aborted June 9, 1927, 146 days after first exposure and 75 days after the last; afterbirth showed characteristic abortion-disease lesions, and was found to be infected with *Br. abortus* as were also the organs and stomach fluid of the fetus; milk negative for *Br. abortus* July 11, 14, and 20 and November 22, 1927, and January 11 and 28, February 25 and 28, and March 3, 1928.

Heifer 1300. Aborted April 4, 1927, 80 days after first exposure and 9 days after the last; afterbirth retained and proved to be infected with *Br. abortus* as were also the organs and stomach fluid of the fetus; and this microorganism was isolated from the lungs, liver, spleen, and stomach fluid of the fetus by cultural methods. Milk proved to be infected April 6 but not infected July 11, 14, and 20.

Heifer 1352. Aborted a fetus, about 7½ months developed, March 22, 1927, 67 days after first exposure and 4 days before the last; afterbirth firmly retained and proved to be infected with *Br. abortus*, as were also the organs and stomach fluid of the fetus; milk infected March 26, April 2, and July 20.

Heifer 1357. Gave birth to full-term calf July 12, 1927, 179 days after first exposure and 108 days after the last; afterbirth passed normally and negative for *Br. abortus;* milk also negative July 14 and 20.

Heifer 1371. Gave birth to apparently normal calf March 26, 1927, 71 days after first exposure and on the day of the last exposure but before it was made;

afterbirth passed normally but showed lesions resembling those of abortion disease and proved to be infected with *Br. abortus;* milk infected April 2 and 6 and July 14, but not infected July 11 and 20 and November 23.

The results of exposing vaccinated and unvaccinated pregnant cows and heifers to *Br. abortus* are shown in condensed form in Table 8.

TABLE 8.—*Comparative resistance to Brucella abortus exposure of pregnant cows and heifers vaccinated after breeding with different strains of Br. abortus and those not vaccinated*

Group No.	Cattle	Strain of vaccine	Abortions or premature calves		Cows producing full-term calves		Cows producing infected or noninfected milk		Cows entirely resisting infection	Full-term calves which lived
			Caused by Br. abortus	Not caused by Br. abortus	Afterbirth infected	Afterbirth not infected	Infected	Noninfected		
	Number		*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*
1	4	Avirulent bovine	1	0	2	1	1	3	25	75
2	[a] 5	Virulent swine	0	0	1	4	[b] 1	4	60	100
3	6	None (control)	3	0	1	2	3	3	33.3	50

[a] Heifer 1372, which calved 22 days after a single exposure, is not included but the animal that replaced her is.

[b] Udder infection caused by vaccination and not by exposure.

EFFECTS OF VACCINATION AND EXPOSURE ON AGGLUTININ CONTENT OF BLOOD

Tables 9, 10, and 11 give the results of agglutination tests made at intervals from the time of vaccination until after the termination of pregnancies. In general the tests were made in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, and 1:800. In a few instances the dilution was carried to 1:1,600 and 1:3,200. In some cases, when it seemed certain that the 1:25 dilution would not furnish additional data, it was omitted as were also the higher dilutions after the data obtained were deemed adequate.

It will be seen in Tables 9 and 10 that agglutinins appeared in the blood of all the vaccinated animals in about a week and had become markedly reduced about 47 days later except in the case of heifer 1358, whose udder became infected with the vaccine.

Attention is called to the close agreement between the blood titers and bacterial findings in Table 10. The titers of the blood of each animal in the group, except the animal whose udder became infected with the vaccine, gradually fell to a low point and did not again appreciably increase.

Table 11 shows that the long-continued exposure had little power to bring out reactions, even in low titer in the two controls that did not become infected, and that the time of appearance of agglutinins after exposure may vary somewhat.

TABLE 9.—*Results of agglutination tests of blood sera of cattle in group 1 (vaccinated with a virulent bovine strain of Brucella abortus during pregnancy) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively* [a]

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1226	Cow 1361	Heifer 1327	Heifer 1367
	1926				
	Nov. 22	-----	-----	-----	s----
	Dec. 13	-----	-----	-----	s----
1926	Dec. 16	-----	-----	s----	s----
	Dec. 20		o+++ps-	++++s-	o++ps--
Dec. 13, vaccinated	Dec. 24	o+++s--	o++++s-	++++++	o+++ps-
	1927				
	Jan. 4	o+++++s	o++++ps-		o++++s-
	Jan. 14	o+++s--	o++ps--	o++++s-	o+++s-
	Jan. 26	o+++s--	o++s---	o+++s--	o++s---
	Feb. 5	o+ps---	o+ps---	o+ps---	o+ps---
	Feb. 17	o++s---	o+s----	s+p----	o+p----
	Feb. 28		ops--	o+s--	o+p----
	Mar. 7	o+++p--	o+s----	o++s---	o+ps---
	Mar. 14	o+++p--	ops----	o+p----	o+s----
	Mar. 21		o++s---	o+p----	ops----
	Mar. 28	o+++p--	o++++--	o+p----	os-----
	Apr. 2	o+++p--	o++++p-	o+p----	s---
Five exposures from Jan. 14 to Mar. 26, 1927, inclusive	Apr. 9	o+++p--	o+++++s	o++++s	+s---
	Apr. 18	o+++p--	o+++++p	o++++p-	
	Apr. 20		[b] o+++++		+s---
	Apr. 26	o+++s--	o++++++	o+++++s	[c] +ps--
	May 10	++p--	o++++++	+++++	
	May 18	+++p-	o++++++	[d] o++++++p	
	June 9	o++p--			
	June 29	[e] o++----			
	July 11	+ps--	o++++s--		
	Oct. 12	++s--	o+++s-		
	1928				
	Jan. 11	o+s---	o+++s-		

[a] Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; -=no agglutination; o=no test was made in this dilution.
[b] Apr. 20, aborted.
[c] Apr 25, calved.
[d] May 24, calved.
[e] June 27, calved.

TABLE 10.—*Results of agglutination tests of blood sera of cattle in group 2 (vaccinated with virulent swine strain of Brucella abortus during pregnancy) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively* [a]

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1364	Heifer 1301	Heifer 1349	Heifer 1351	Heifer 1358	Heifer 1372
	1926 Nov. 22	-----	-----	-----	-----	-----	-----
Dec. 8, 1926, vaccinated	Dec. 9				-----		
	Dec. 11				s----		
	Dec. 13	-----	-----	-----		-----	-----
	Dec. 16	-----	-----	-----	++++p		
Dec. 13, 1926, vaccinated	Dec. 20	o+ps---	+++ps-	o++p---	o++++p-	o++s---	o+++s--
	Dec. 24	o+++s--	+++++p	o+++ps-		o+++s--	o++++ps
	1927 Jan. 4	o++++p-	o+++++p	o++++s-	o++++--	o++++++	o++++p-
	Jan. 14	o+++ps-	o+++++s	o++p---		o+++++s	o+++s--
	Jan. 22				o+++p--		
	Jan. 26	o+++s--	o++++s-	o++-----		o++++s-	o+ps---
	Feb. 5		o+++p--	o+s-----	o+s-----	o+++p--	[b] o+ps---
	Feb. 17	o++s---	o+++s--	op-----	o+s-----	o++++--	o+p-----
	Feb. 28	o+ps---		os-----	o+s-----	o+++p--	ops-----
	Mar. 7	o++s---	o+++s--	ops-----		o++++s-	o+p-----
	Mar. 8				o+s-----		
	Mar. 14	ops-----	o+++s--	o+s-----	os-----	o+++p--	ops-----
	Mar. 21	ops-----		ops-----	os-----	o++++--	
	Mar. 28	ops-----	o++s---	ops-----	os-----	o++++ps-	o+p
	Apr. 2	ops-----	opps---	+s-----	os----'-	o++++s-	o+s-----
Five exposures from Jan. 14 to Mar. 26, 1927, inclusive	Apr. 9	ps----	+++p-	+s-----	+p----	o++++s-	++s----
	Apr. 18	++s--	o+ps---	ps----	+s----	o+++++--	
	Apr. 26	s------	o+ps---	ps----	+ps---	o+++++ps	++p--
	May 5				ps----	[c] o++++pp	
	May 10	+s----	o+p-----	ps----	+s-----	o++++++s-	o++----
	May 18	ps----	++s--	[d] ps----	ps----	o++++ps	ps----
	June 2				ps----		
	June 9	pss--	pps--		ps----		
	June 10	[e] +ps					
	June 25				[f] ps-----		
	June 29		[g] ops-----				
	July 11	+p----	+ps-----	ps----	+s-----	o++++p--	ps----
	Oct. 12	s-------	+p----	+s----	ps----	o++p--	
	1928 Jan. 11	ps----	+p----	+ps--	ps----	o++++s-	+s----

[a] Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; -=no agglutination; o=no test made in this dilution.
[b] Feb. 5, calved. [c] May 5, calved. [d] May 18, calved. [e] June 10, calved. [f] June 24, calved. [g] June 29, calved.

TABLE 11.—*Results of agglutination tests of blood sera of [illegible]*

TABLE 11.—*Results of agglutination tests of blood sera of cattle in group 3 (controls) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively* [a]

Date of first and last exposure	Date of agglutination test	Cow 1174	Cow 1338	Heifer 1300	Heifer 1352	Heifer 1357	Heifer 1371
Five exposures from Jan. 14 to Mar. 26, 1927, inclusive	1926 Nov. 22	-----	-----	-----	-----	-----	s-----
	1927 Jan. 4	-----	-----	-----	-----	-----	
	Jan. 14		0------	0------	-----	-----	p-----
	Jan. 20	0-----			0------	0------	0s----
	Jan. 26	0-----	0------	0------	0------	0------	
	Feb. 5	0-----	0------	0------	0------	0------	0s-----
	Feb. 17	0-----	0------	0------	0------	0------	0s-----
	Feb. 23	-----	-----	-----	0------	0------	0------
	Feb. 28	0-----	0------	0------	-----	-----	-----
	Mar. 7	0-----	0------	0++++ps	0------	0------	0------
	Mar. 14	0-----	0------	0++++p-	0------	0s-----	0s-----
	Mar. 21	0-----	0------	0+++ps-	0------	0------	0s-----
	Mar. 26				[b] 0+p----	0------	0+p----
	Mar. 28	0-----	0------	0++++--	0+p----		[c] 0ps----
	Mar. 30				0+++++s-	0------	0ps----
	Apr. 2	-----	-----	[d] 0+++++--	0+++++s-	s-----	0++----
	Apr. 9	s-----	s-----	0+++++--	0+++++p-	s-----	0++ps
	Apr. 18	s-----	s-----	0++++++	0+++++ps	s-----	0+++p--
	Apr. 26	-----	s-----	0++++++p-	0+++++s-	-----	0++ps
	May 10	s-----	ps----	0++++ps-	0+++++p-	s-----	0++++s
	May 18	-----	ps----	0++++++s	0++++p-	-----	0+++p--
	June 1	-----				s-----	
	June 9	-----	[e] 0++++p--			s-----	
	June 15	[f] s-----				-----	
	June 27					s-----	0++ps-
	July 11	-----	0+++++p-	0++pp-	0+p------	[g] s-----	
	July 12						
	Sept. 13		0+++++s-				
	Oct. 5		0++++p-				
	Oct. 12	-----	0++++s--		0+p----	-----	0+++--
	Nov. 22				0++p--		
	Dec. 16	-----					
	1928 Jan. 11		0+++p--			-----	0+++ps
	Mar. 3						0++++-

[a] Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; -=no agglutination; 0=no test made in this dilution.
[b] Mar. 22, aborted. [c] Mar. 26, calved. [d] Apr. 4, aborted. [e] June 9, aborted. [f] June 15, calved. [g] July 12, calved.

SUMMARY AND CONCLUSIONS

Experiments were made to determine the efficacy of three strains of *Brucella abortus* as immunizing agents against infectious abortion. The strains tested were an avirulent bovine strain, a virulent swine and a virulent bovine strain. The results of these experiments indicate that the strain of bovine *Br. abortus* avirulent for guinea pigs or the virulent swine strain, when administered to cows and heifers three to five and one-half months before service, afforded only slight protection against repeated exposure to *Br. abortus* as compared with that induced by the virulent bovine strain. When given after conception the avirulent bovine strain seemed to increase resistance to the progress of the disease and the swine strain appeared to give a definite immunity. However, the latter strain is likely to infect the udder for long periods and hence its use for vaccinating cattle, because the swine strain of *Br. abortus* is generally believed to be more dangerous to human health than the bovine, is out of the question.

These findings throw light on the relation of swine strains of *Br. abortus* bovine and show the danger of infecting the udder with a swine strain of *Br. abortus* if it should, by accident, find its way into vaccine.

Neither the avirulent bovine nor the virulent swine strain of vaccine, administered from three to five and one-half months before breeding, produced much more than a slightly greater resistance to the long-continued severe exposure given than that possessed by the controls; nor did either of the vaccines prevent the udders from becoming infected. In one instance the swine strain of vaccine caused udder infection.

A vaccine prepared from virulent bovine *Br. abortus* afforded a marked resistance to abortion and uterine infection under the same conditions as those of the two previously mentioned strains but either did not prevent udder infection or was the direct cause of it in half the animals that completed the experiment. Udder infection, however, was greater among the controls. The accidental use of this vaccine on a pregnant heifer caused her to abort.

Brucella abortus of the swine type, when used as vaccine before conception, may invade the udder and associated lymph glands and persist there for at least one and one-third years, and if administered to pregnant cattle may also invade the udder and persist there, without appreciable change in character, for at least four years.

DISTRIBUTION OF THE COTTON ROOT-ROT FUNGUS IN SOIL AND IN PLANT TISSUES IN RELATION TO CONTROL BY DISINFECTANTS [1]

By C. J. KING, *Agronomist*, and CLAUDE HOPE, *Junior Horticulturist, Division of Cotton, Rubber, and Other Tropical Plants, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

The root-rot disease caused by the fungus *Phymatotrichum omnivorum* (Shear) Dug. is responsible for heavy losses in many of the economic plants grown in the Southwestern States. This fungus has a wide range of host plants but is less destructive to some species than to others. Some species possess powers of resistance apparently because of some peculiar histological structure of the roots, and others contain certain chemical substances which seem to repel the invasion of the fungus. Some of the trees and shrubs, because of their habit of developing extensive root systems, are able to resist the disease after attaining some degree of maturity. Even those that are highly susceptible often live for several months after becoming infected, and certain individuals may live for years because of some unusual distribution of their roots, which allows part of them to escape infection. On account of these differences in resistive powers, it is not always possible to determine from the appearance of the tops whether the plants are infected.

It is often desirable for the plant grower or the investigator to determine the actual limits of areas that are infested with the root-rot fungus. Where field crops such as alfalfa or cotton are grown continuously for several years this may be accomplished fairly accurately by mapping periodically the areas of dead plants. In plantings of trees and shrubs or in fallow land, however, different methods are required.

Incidental to an attempt to eradicate the root-rot fungus by the use of formaldehyde in an isolated area of approximately 5 acres, near Indio, Calif., in 1930, some information was secured which would appear to be of value in relation to any control methods or investigations that are undertaken. The practicability of mapping infested areas was indicated even where no infected plants were present to serve as indicators. It was also brought out that hyphae that had gained entry into the interior tissues of large woody roots were afforded protection against disinfectants for a long period.

The discovery in 1928 of a sclerotium stage in the life history of the root-rot fungus led to an intensified study on the part of several investigators to determine the character of the sclerotial structures and their relation to the dissemination of the disease and its carry-over from one season to another.

The production of sclerotia in pure cultures at Sacaton, Ariz., in 1928, was first reported by King and Loomis (*3*) [3] in 1929. Neal (*6*)

[1] Received for publication Mar. 5, 1932; issued December, 1932.

[2] The writers are indebted to H. F. Loomis, associate agronomist, and Orlan Parker, assistant scientific aide, of this division, and to F. A. Thackery, senior agriculturist, and Dewey C. Moore, scientific aid, of the Division of Horticultural Crops and Diseases, for valuable assistance in conducting these investigations.

[3] Reference is made by number (italic) to Literature Cited, p. 740.

and later Dana (*1*) and Taubenhaus and Ezekiel (*8*) reported the finding of sclerotia under natural conditions in the soil in Texas. Further contributions by Taubenhaus and Ezekiel (*9*), Dana (*2*), and King, Loomis, and Hope (*4*) have furnished additional information on the properties of sclerotia. Some investigations conducted in Arizona in 1930 and 1931 on the distribution of sclerotia in soil where various types of host plants were grown are reported as a part of this paper.

METHOD OF MAPPING INFESTED AREAS

Soon after the identification of the root-rot fungus in an isolated area near Indio, Calif., in 1928, some preliminary measures were taken to prevent further spread.

Before an adequate survey was made to delimit the infested area, trenches were dug a few feet outside the supposed limits of the infested spots, with the idea that they might serve as barriers against further spread of the fungus. Only such areas were included within the trenches as showed the disease by aboveground symptoms on the susceptible plants.

It soon became apparent that many of the trenches were within the infested area. When moisture conditions in the trenches became favorable, spore mats appeared in many of them, even at some distance from growing plants. (Fig. 1.) On part of the area a second line of trenches was later dug farther out, but in these also spore mats appeared in some places. Although the open trenches were not infallible guides to the presence of the disease where no plants were present, their value in this respect was well demonstrated.

When it was decided to treat the entire infested area with a disinfectant, a survey was made by excavating and examining the roots of plants in areas where the presence of the disease was suspected. The inspectors were aided in this work by the fact that the entire area of about 5 acres was more or less permeated by the roots of susceptible trees. In parts of the area, the trees themselves were still standing, and in other places they had been removed some months or even years previously. It was possible to find living or dead roots in almost every excavation.

By making a large number of excavations and charting each spot in which infected roots were found, it was possible to obtain a fairly good idea of the limits of the disease; it was discovered that the infestation involved an almost continuous area instead of being confined to disconnected spots as indicated by the dead trees and crop plants.

Two parallel rows of large pistache trees (*Pistacia atlantica* Desf.) showed no evidence of disease aboveground, but excavations proved that two trees at each end of the avenue had infected roots, parts of which extended into an adjacent date orchard. Similar evidence was found along a hedge of large athel trees (*Tamarix aphylla* (L.) Karst.). No direct evidence was found that the athel roots were attacked, but the presence of the fungus on the surface of some of the roots and the occurrence of the disease in test crops in adjacent land beyond the athel hedge indicated that the roots may have served as a host. The roots of other species of Tamarix in a mixed planting which formed a hedge about 100 feet long at the northwest corner of the garden, were found to be seriously infected, although no trees had died. Large portions of the roots of these trees throughout the length of the

FIGURE 1.—Conidial mats of the root-rot fungus forming in a trench near the margin of an infested orchard near Indio, Calif.

hedge were rotted away and showed many fragmentary strands of Phymatotrichum.

Under the conditions at Indio the fungus apparently may exist in an active state for a long time on the roots that are left in the soil after the aerial parts are removed. The experience in this case suggests the great importance of learning the history of an infested area before planning experiments or attempting control measures, and indicates the feasibility of detecting the presence of the disease in the absence of indicator plants by a proper survey and examination of the roots within the suspected area.

CONDITIONS INFLUENCING THE EFFECTIVENESS OF DISINFECTANTS

Experiments conducted in an attempt to eradicate spots of root rot by applying disinfectants to the soil brought out the fact that the mycelium that had penetrated into the inner tissues of roots was protected against disinfectants and that this protection offered one of the most serious obstacles to the success of control measures. The existence of protected hyphae was first recognized in making cultures of the root-rot fungus from interior tissues of large roots at Indio, Calif.

Where the soil is permeable to solutions, sclerotia and exposed strands are readily destroyed by formaldehyde (*4*). Laboratory tests showed that the germinating power of sclerotia of average size (about 2 mm) was destroyed when they were immersed in a 1 per cent solution of commercial formaldehyde (formalin) for about 45 minutes. The sclerotia were likewise killed after 3 hours when placed 6 inches deep in cans of soil that had been recently treated with a 1 per cent solution of formalin.

On January 29, 1931, about 70 sclerotia, from 2 to 4 mm in diameter, were removed from four or five borings in part of the area at Indio that had been treated about 10 months previously with a 1¼ per cent solution of formalin. The majority of these sclerotia were soft and shrunken and in a state of decomposition. About 20 of the largest and most turgid individuals were tested for germination, but all of them failed to grow. On June 2, 1931, 118 sclerotia from the treated area were examined, and all proved to be dead; most of them were badly decayed.

The mycelium that had penetrated the tissues of plant roots, however, was not so readily destroyed. A period of 6 hours was required to kill the hyphae in sections of fig (*Ficus carica* L.) and chinaberry (*Melia azedarach* L.) roots, one-half to three-fourths inch in diameter, immersed in a 1¼ per cent solution of formalin. A period of 12 hours was required to kill the mycelium in ½-inch cotton roots placed in a soil that had been treated with a 1 per cent solution of formalin.

At Indio the attempt to eradicate the root-rot fungus was carried out under the direction of F. A. Thackery in cooperation with the California State Department of Agriculture (*5*). The method consisted in introducing a 1¼ per cent formalin solution under pressure by means of injector nozzles on the ends of ⅜-inch gas pipes, which were inserted gradually into the soil to a depth of 6 feet. (Fig. 2.) The injections were made 1 foot apart, and 6 gallons of solution was introduced at each injection.

The soil in the infested area consists of a very fine sand that extends to great depths; its permeable character makes it especially favorable for the uniform distribution of the solution.

In order to obtain some idea of the effectiveness of the treatment against mycelium that had penetrated the inner tissues of roots in the soil, large numbers of root sections of fig, pistache, and chinaberry, from three-eighths to 1 inch in diameter, were sterilized in an autoclave and infected with the root-rot fungus. After the fungus had developed and maintained a profuse growth for about 5 days, each section was tagged, some were wrapped in filter paper, and all of them were inserted in the soil at depths of from 1 to 7 feet. (Fig. 3.) The formalin solution was injected over them in the regular manner, after which specimens were removed at intervals of 1 to 30 days and tested to determine whether the fungus remained alive. A revived growth of the fungus resulted from most of the root sections removed during the first 12 days after treatment, but only 25 to 30 per cent of those that were left in the soil from 12 to 30 days showed further root-rot growth.

FIGURE 2.—Introducing formalin solution into an infested area in an effort to eradicate the root-rot fungus

In another test, fig and chinaberry roots 1½ to 2 inches in diameter were cut into sections about 3 inches long. A hole about 1 inch deep was made in one end of each with a ⅜-inch drill bit. After the sections had been sterilized, root-rot infected root tissues were inserted in the holes, which were then stoppered tightly with corks, and the stoppered ends sealed with paraffin. (Fig. 4.) They were then inserted in the soil in the manner described above and the formaldehyde treatment applied.

Specimens were removed from the soil for examination at intervals varying from 10 to 30 days. The fungus was still alive in all the specimens; in most cases it was found that the mycelium had grown through the conducting vessels of the roots and had escaped at the unsealed end of each section, where it developed a dense growth that in some cases almost covered the entire root section. Contaminations were found on some of the sections, but the root-rot mycelium was the predominating growth.

FIGURE 3.—Cultures of the root-rot fungus on roots of chinaberry and of fig. These were placed in the soil at various depths to test the effects of the formalin solution

This behavior is in accord with the observations of Peltier, King, and Samson (7), who reported the presence of hyphae in the outer xylem vessels of alfalfa roots 50 cm beyond the diseased portions. Under natural conditions it is quite possible that hyphae find their way through deep lesions or wounds into the conducting vessels of many woody roots and extend far into the healthy tissues. Under such conditions they would probably be protected for long periods against any disinfectants that might be applied to the soil.

On January 29, 1931, a large number of roots and root fragments were exhumed in the area that had received the formaldehyde treatment about 10 months previously. For the most part they were in a soggy condition unfavorable to the growth of the fungus. Fragments of strands were still visible on the surface of some, but these appeared to be dead and in a state of decomposition.

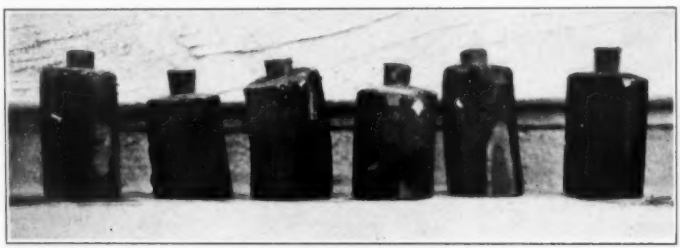

FIGURE 4.—Roots of fig and chinaberry with root-rot inoculum stoppered inside the tissues. These were placed in the soil at various depths to test the effects of the formalin solution

VIABILITY AND PROPAGATIVE POWERS OF SCLEROTIA

In a previous publication, King, Loomis, and Hope (4) reported successful inoculation of cotton plants with sclerotia 11 months old and described a budding process by which the viability of sclerotial masses was believed to be prolonged.

On May 6, 1931, a test was made to determine whether any of the sclerotia were viable in a culture jar of sand and cotton roots that had been kept in the laboratory since the sclerotia were first noted on October 29, 1928, over two and one half years before. Water had

been added to this jar about eight months after the culture was prepared, but after that it had received no attention. A little moisture was present in the sand at the time the sclerotia were tested. A few of the sclerotia showed signs of decomposition, but the majority were still well preserved. Thirty-one of the latter were placed on moist filter paper in Petri dishes and incubated. Within a few days all of them had germinated and produced a vigorously growing mycelium.

In December, 1930, a test was conducted to determine whether sections and fragments of sclerotia possessed the powers of germination and growth shown by entire individuals. Taubenhaus and Ezekiel (*8*) reported that the same sclerotia would produce growth as many as five times after the previous growth had been removed, and it seemed possible that hyphal growth might issue from almost any part of the structures. Accordingly, 40 large, well-developed simple sclerotia that had recently been taken from infested soil were selected for the test. After they had been washed in mercuric chloride solution and sterile water they were divided into three lots. From each of the 20 sclerotia in lot 1 the thin outside coat was carefully cut away, so that as little as possible of the inner tissue was removed in the process. From the 5 individuals in lot 2, thin cross sections were made with a scalpel. Ten of these sections and 42 of the fragments removed from the first group were then selected at random for testing.

Lot 3, consisting of the remaining 15 untreated sclerotia, was used as a check. All three lots were than placed on sterilized moist filter paper in Petri dishes and incubated. Within a few days, typical root-rot strands had grown from 19 of the 20 sclerotia from which the outside tissue had been removed, the 52 fragments and sections had germinated, and the 15 sclerotia used as a check had produced vigorous growth. (Fig. 5.) The growth from the fragments and "peeled" sclerotia was hardly as vigorous as that from the unmutilated ones, but undoubtedly there would have been no difficulty in establishing cultures or in obtaining infection from this material. Later this experiment was repeated, and the results were practically the same.

DISTRIBUTION OF SCLEROTIA IN INFESTED AREAS

In 1928 repeated search was made for sclerotia in the soil of infested areas at Sacaton, Ariz. After several months some were found, but only in parts of some areas and not at all in others. In most areas the color of the sclerotia was so similar to many of the soil particles that it was not practicable to distinguish between them in examining samples of dry soil by ordinary methods of dissection. A method was devised which made it practicable to separate the sclerotia from the soil particles so that they could be counted and estimates made of the number in an infected area.

The procedure was as follows: Soil samples of equal volume were removed from definite zones at various depths by means of a trowel or post-hole auger. Each sample was placed in the top of a set of two sieves, each 12 inches square. The bottom of the upper sieve was made of hardware cloth of ¼-inch mesh, and that of the lower sieve of 20-mesh screen wire. (Fig. 6, A.) The finer soil particles were forced through the screens by water under pressure from a hose nozzle, leaving the larger particles, together with root fragments, on the top

FIGURE 5.—Germination of root-rot sclerotia: A, 20 sclerotia developing vigorous mycelial growth after removal of outside covering; B, sections of inside tissues and fragments of the covering or "peel" producing growth of root-rot mycelium; C, 15 whole sclerotia germinating to produce growth of root-rot mycelium

screen, and the sclerotia, with coarse sand and other débris, on the bottom screen. The sclerotia could then be picked out easily with forceps. (Fig. 6, B.)

When this method was first adopted, the soil was removed in layers 4 inches thick and 12 inches square. In an infested cotton field examined in August, 1930, the 4-inch blocks were obtained in duplicate to a depth of 24 inches, and the two from the same depth were combined to form two-thirds of a cubic foot of soil. This was placed on the screens, and the sclerotia were separated by the washing process. The number of sclerotia contained in each 4-inch layer taken at depths of from 8 to 24 inches, was as follows:

Depth in inches	Number of sclerotia
4–8	0
8–12	11
12–16	226
16–20	632
20–24	152

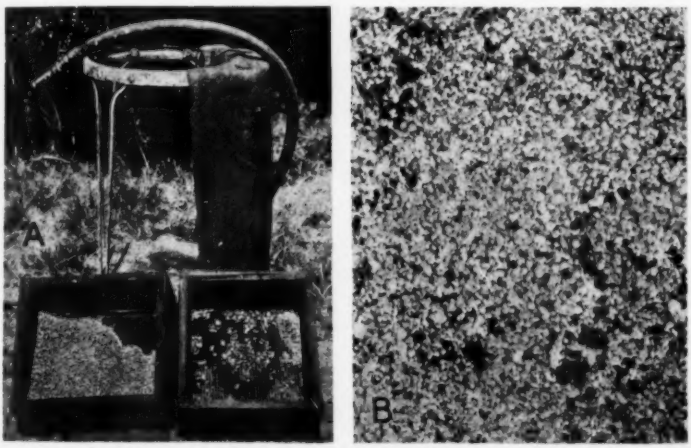

FIGURE 6.—A, Equipment used in separating root-rot sclerotia from the soil to determine their size and number in infested areas; B, part of residue of coarse sand and sclerotia left on bottom screen after a 13-pound sample of infested soil had been washed through the screens. The sclerotia are usually much darker than the soil particles and can be readily picked out from the residue with forceps. B about one-half natural size

So many sclerotia were found in the layer between the depths of 20 and 24 inches that probably other sclerotia would have been found below the latter depth if the excavations had been carried farther.

In cultivated land, sclerotia were seldom found at a depth of less than 4 to 6 inches; therefore in most of the sampling operations, the topsoil to that depth was discarded.

In January, 1931, several borings were made for soil samples in an infested area in an alfalfa field near Coolidge, Ariz. The soil in this area is of the Mohave sandy loam type, containing much gravel and a high proportion of calcareous material locally known as caliche. The spot selected was almost semicircular in shape and about 20½ feet in longest diameter. It was bordered by a fence separating two fields of alfalfa. A few of the alfalfa plants across the fence from the spot showed signs of infection, but the limits of infestation were

not well defined. Six borings (Nos. 1 to 6) were made with a 7-inch posthole auger on the periphery of the circular spot along the margin of dead plants. (Fig. 7.) Hole 9 was located near the center, holes 7 and 8 midway between the center and the periphery, and hole 10 among live plants a few inches beyond the fence in the other field.

On a later date three holes, designated as 1–A, 2–A, and 3–A, were made outside the margin of dead plants opposite holes 1, 2, and 3, respectively, so that their centers were 10 inches from the centers of the holes previously made. Three others, designated as 1–B, 2–B, and 3–B, were located still farther out among the living plants, so

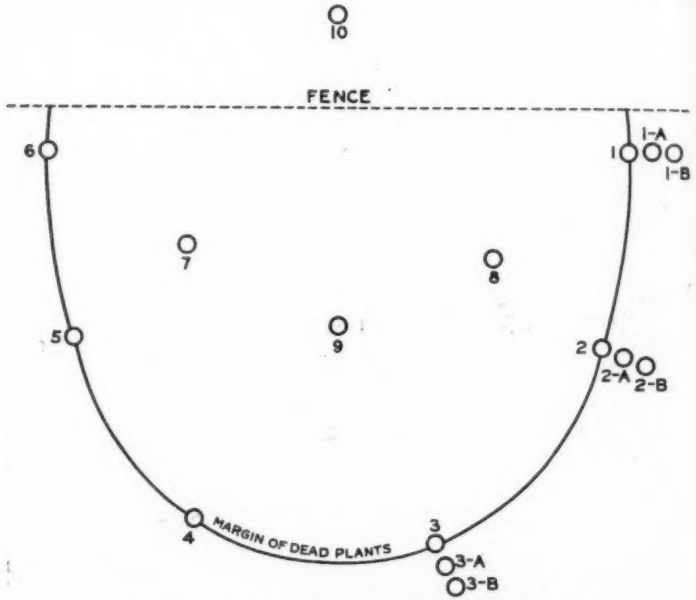

FIGURE 7.—Diagram of a spot of root-rot infection in alfalfa, near Coolidge, Ariz., showing the location of the holes from which soil samples containing sclerotia were removed with a 7-inch posthole auger. Six holes (Nos. 1–6) were located along the margin of dead plants; hole 9, near the center; holes 7 and 8, midway between the center and the periphery; hole 10 among live plants; holes 1–A and 1–B; 2–A and 2–B, and 3–A and 3–B, among live plants, with centers 10 and 20 inches from the centers of holes 1, 2, and 3, respectively. (Table 1.) Scale, 1 inch=6 feet

that their centers were 20 inches beyond the centers of holes 1, 2, and 3 on the margin. After the top 6 inches had been discarded, the soil from each 6-inch depth, representing about 13 pounds of soil, was placed in separate containers and conveyed to the laboratory for separation of the sclerotia by the washing process. Some of the holes were dug only 30 inches deep, but others were sampled to a depth of 42 inches, and holes 2, 3, and 9 were 54 inches deep. The number of sclerotia found at different 6-inch depths in these first 10 holes that were dug is given in Table 1. Since only 12 were found in the holes that were dug later, outside the area of dead plants, the results are not included in the table. These occurred at a depth of 6 to 18 inches in hole 3–A.

TABLE 1.—*Distribution of root-rot sclerotia in an infected spot of alfalfa near Coolidge, Ariz.*

Depth (inches)	Number of sclerotia in hole No. [a] —										
	1	2	3	4	5	6	7	8	9	10	Total
6-12	53	58	5	287	0	1	2	101	1	2	510
12-18	51	305	[b] 212	163	12	1	0	171	129	13	1,057
18-24	6	14	15	17	6	0	0	211	690	4	963
24-30	18	1	7	2	1	0	1	157	282	6	475
30-36	5	1	0	.....	.....	.....	.....	.....	161	.....	167
36-42	0	0	0	.....	.....	.....	.....	.....	140	.....	140
42-48	.....	0	0	.....	.....	.....	.....	.....	0	.....	0
48-54	.....	0	0	.....	.....	.....	.....	.....	4	.....	4
Total	133	379	239	469	19	2	3	640	1,407	25	3,316

[a] See fig. 7.
[b] 5 of these sclerotia were dead.

While some sclerotia were found in each of the 10 holes, they were unevenly distributed throughout the soil. The greatest number was obtained in hole 9, which was in the center of the spot. The eight samples from this hole gave a total of 1,407 sclerotia. These were darker in color than most of those from other holes, indicating that they were older, but they were all viable. Judging by the size of the spot, they must have developed at least a year previously, when the disease first became active at the center. From the large number of sclerotia present in some holes at depths of 6 to 12 inches, it is probable that there were a few in the top 6 inches of soil that was discarded. In all the borings the sclerotia were most abundant at depths of 6 to 24 inches. In hole 9 a considerable number were found at a depth of 30 to 42 inches, and four occurred at a depth of 48 to 54 inches.

During April and May, 1931, soil samples were taken from several parts of an infested area in a Bermuda-grass lawn at the United States Field Station at Sacaton, Ariz. In July, 1930, an 11-year-old Chinese elm (*Ulmus pumila* L.) located in this area died suddenly from root rot and was dug out. The main laterals were cut a few inches from the taproot and were left in the soil. Except for another Chinese elm, located about 15 feet distant, there were no other susceptible plants near by. The soil in this area is a Gila fine sand that is fairly uniform throughout the depth sampled. In sampling, the top 3 inches, consisting largely of Bermuda-grass sod, was discarded. The soil from a depth of 3 to 6 inches comprised the first sample; below 6 inches, the samples were taken in 6-inch layers.

In most of the borings the samples were taken to a depth of 3 to 7 feet with a 7-inch posthole auger. Samples from lower depths were taken with a 6-inch posthole auger. This was done to prevent the sclerotia from being dislodged from the sides of the holes at upper levels and thus becoming mixed with samples from greater depths. As a further precaution, the 6-inch auger was operated on the inside of an iron pipe having a diameter slightly larger than that of the auger. As the depth of the hole increased the pipe was pressed gradually into the soil just behind the cutting edge of the auger, and the samples were drawn upward through the pipe without making contact with the soil on the sides of the hole.

A diagram showing the location of all the holes with reference to the tree is given in Figure 8. The numbers of sclerotia found at different depths in the nine holes made in this area are given in Table 2.

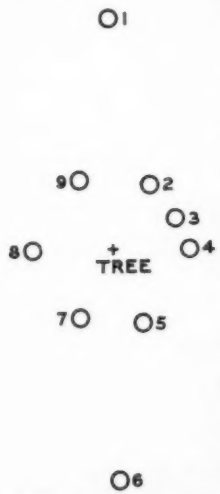

FIGURE 8.—Diagram showing position of holes (Nos. 1-9), from which soil samples containing root-rot sclerotia were obtained with a posthole auger, with reference to the location of a Chinese elm that had died from root rot. (Table 2.) Scale, 1 inch=6 feet

In this, as in the other areas sampled, the sclerotia were unevenly distributed and appeared to concentrate more or less in pockets of soil.

In holes 5 and 8 the sclerotia were most abundant at a depth of 6 to 18 inches; in hole 9, at a depth of 18 to 30 inches, and in hole 1 at a depth of 30 to 42 inches. In hole 2 very few were obtained above a depth of 36 inches, but a large number occurred at greater depths. No sclerotia were found in hole 6, which was located 8 feet south of the tree, but 892 were obtained from hole 1, 8 feet north of the tree.

In holes 5 and 9 a few sclerotia were found in the samples removed from a depth of 84 to 90 inches. Their occurrence at practically all the 6-inch levels above that depth indicates that their presence at this great depth was not the result of accident in sampling. Live root-rot strands on diseased roots were also found at that level, and this gave further weight to the evidence that the sclerotia actually developed 84 to 90 inches below the surface of the soil.

It was found that a sample of soil taken from the lower levels with the 6-inch auger weighed about 13 pounds, which was about the same as a sample of the same thickness taken near the surface with a 7-inch auger.

TABLE 2.—*Distribution of root-rot sclerotia in an infested area where a Chinese elm tree had died from the disease at Sacaton, Ariz., in 1930*

Depth (inches)	Number of sclerotia in hole No.[a]—									
	1	2	3	4	5	6	7	8	9	Total
3–6	0	0	0	-------	0	0	13	0	-------	13
6–12	12	0	0	4	838	0	36	882	5	1,777
12–18	26	2	[b] 3	10	81	0	142	744	61	1,069
18–24	75	18	[c] 49	64	33	0	168	48	774	1,229
24–30	106	5	71	38	18	0	21	6	856	1,121
30–36	277	0	53	19	98	0	8	3	108	566
36–42	346	77	[d] 3	809	28	0	32	6	154	1,455
42–48	[d] 39	338	1	141	11	0	59	3	69	661
48–54	11	12	5	5	11	0	55	2	14	115
54–60	0	32	4	9	8	0	7	3	4	67
60–66	0	58	0	[d] 0	3	0	7	[d] [e] 4	3	75
66–72	0	18	0	0	4	0	3	0	0	25
72–78	0	3	0	0	1	-------	3	0	3	10
78–84	0	2	0	0	2	-------	11	0	1	16
84–90	-------	[d] 0	-------	-------	[d] 1	-------	[d] 0	-------	[b] [d] 3	4
90–96	-------	0	-------	-------	0	-------	0	-------	0	0
96–102	-------	-------	-------	-------	0	-------	-------	-------	0	0
Total	892	565	189	1,099	1,137	0	565	1,701	2,055	8,203

[a] See Fig. 8.
[b] Two of these sclerotia were dead.
[c] Seven of these sclerotia were dead.
[d] The sheet-iron pipe and 6-inch auger were used in removing this and lower samples from this hole.
[e] Three of these sclerotia were dead.

Of the nine holes that were made in sampling, seven were located roughly in a circle approximately 2½ feet from a center representing the site of the tree trunk. One hole (No. 1) was made 8 feet north and another hole (No. 6) 8 feet south of the same central point.

The total number of sclerotia obtained in all samples from the nine holes was 8,203. Of these, 7,311 were obtained from the seven holes (Nos. 2, 3, 4, 5, 7, 8, and 9) located on the periphery of a circle with a radius of approximately 30 inches. (Fig. 8.) Using the average of

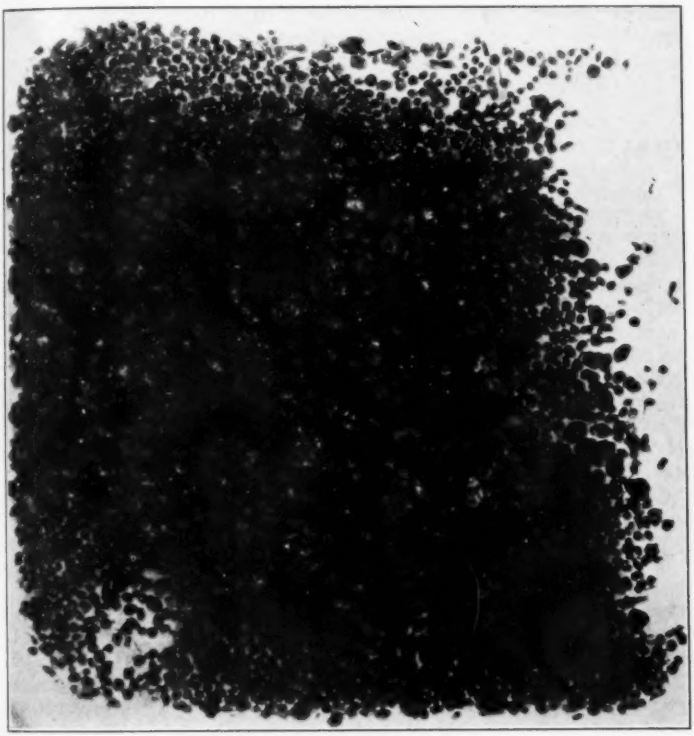

FIGURE 9.—A mass of sclerotia (approximately 5,000) obtained from soil samples removed in nine borings in an area where a Chinese elm had died from root rot. Natural size

these holes as a basis, it is estimated that there were about 100,000 sclerotia within this circle. A mass of about 5,000 sclerotia is shown in Figure 9.

The amount of soil examined was approximately 1,865 pounds. It was estimated that 1 cubic foot weighed 100 pounds, and if it is assumed that the root system of the tree occupied an area of 400 square feet and extended to a depth of 6 feet and that the entire volume harbored a population of sclerotia proportionate to that in the samples, the number of sclerotia involved would be 1,056,616. On this basis, the number of sclerotia in an acre would be 114,956,584.

It has been learned from experience in conducting germination tests with sclerotia that their color grows darker with age and that those which have become soft or flaccid will not germinate. Of the 8,203 sclerotia obtained from the nine borings, 12 were soft and so disintegrated that no test was considered necessary to prove that they were not viable. A large number of the others, including some of the darkest specimens, were tested, and all germinated.

Since the tree was killed about nine months previously and the area had doubtless been infested for some time before that, it is evident that some of the sclerotia must have been at least a year or more old when tested. It would appear, therefore, that they do not lose their viability very rapidly under natural conditions in the soil.

SUMMARY AND CONCLUSIONS

In an attempt to fix the limits of an area infested with the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Dug., near Indio, Calif., methods were developed which served as guides in detecting the presence of the fungus even where no indicator plants were present.

When conditions of moisture were favorable, conidial mats of the fungus formed in great abundance in open trenches that transected the infested area. Numerous trenches dug near the supposed margins of the infested area aided in locating the actual limits of the disease.

In areas where the roots of susceptible plants were abundant, a fairly good idea of the limits of the disease was obtained by making numerous excavations throughout the area and inspecting the root fragments for the presence of the fungus.

It was found that the roots of several large pistache trees (*Pistacia atlantica*) were infected, although the trees showed no symptoms of the disease above ground. Root-rot strands were found on the surface of some of the roots of large athel trees (*Tamarix aphylla*), but no injury to the roots was observed. In other species of Tamarix the roots showed extensive damage from the fungus, but the trees remained alive.

It is evident that the root-rot fungus is able to exist in an active state for a long period on buried roots that remain in the soil after trees have died or have been removed.

The root-rot mycelium that had penetrated into the inner tissues of large roots was protected for a long period from disinfectants that readily destroyed exposed strands and sclerotia.

In attempting to eradicate the fungus from the area near Indio, a 1¼ per cent solution of formalin (commercial formaldehyde) was injected into the soil, by pressure through ⅜-inch pipes, to a depth of 6 feet, at the rate of 1 gallon per cubic foot. To test the effectiveness of this treatment, pure cultures of the root-rot fungus growing on sterilized sections of fig, chinaberry, and pistache roots three-eighths of an inch to 1 inch in diameter were buried from 1 to 7 feet deep in the soil, and the formalin solution was applied over them in the usual way. A majority of the root specimens that were removed from the soil and tested during the first 12 days after the formaldehyde treatment showed a renewed growth of the fungus, but only 25 to 30 per cent of those that remained in the soil 12 to 30 days showed further root-rot growth.

In other tests root-rot inoculum was placed on the inside of steam-sterilized sections of fig and chinaberry roots 1½ to 2 inches in diameter and 3 inches long. These were sealed at one end and buried in the soil at depths of 1 to 7 feet immediately before the formaldehyde treatment was applied. When specimens were removed and examined 30 days after treatment, the inoculum in all the sections was alive, and in some specimens the mycelium had grown through the conducting vessels and partly covered the outside tissue.

Examination of a large number of roots and root fragments removed from the formaldehyde-treated area, about 10 months after the treatment, indicated that all the mycelium on the outside tissues was dead.

In soil samples taken from the formaldehyde-treated area, about 10 months after the treatment, 70 root-rot sclerotia were obtained; in other samples, taken 12 months after the treatment, 118 were obtained. All these were found to be dead.

Root-rot sclerotia from a culture kept in the laboratory for two and one-half years germinated and produced a vigorously growing mycelium.

Laboratory tests showed that all parts of a root-rot sclerotium are capable of producing mycelium. Specimens from which the outer coats had been removed, fragments of the outer coat, and thin cross sections of sclerotia, all produced mycelial growth.

In order to facilitate the separation of sclerotia from the soil of infested areas a sieve made of ¼-inch mesh hardware cloth was placed over another sieve of 20-mesh screen wire. The soil was washed through them, leaving the coarsest soil and root material in the top sieve, and the sclerotia and some of the coarse material in the bottom sieve.

In 1931 a small infested area in an alfalfa field near Coolidge, Ariz., and another on a Bermuda-grass lawn at Sacaton, Ariz., where a Chinese elm had been killed the previous year, were sampled with a posthole auger, and the sclerotia were separated from the soil by the washing process and counted.

In the Coolidge area the sclerotia were most abundant at a depth of 6 to 24 inches, but four were found 48 to 54 inches below the surface. In the Sacaton area the sclerotia were more concentrated at various depths between 6 and 42 inches, but a few were found at a depth of 84 to 90 inches.

It is apparent that the distribution of sclerotia is very uneven, both vertically and horizontally, in infested soils.

In soil samples taken from nine borings in an area where a Chinese elm had died, 8,203 sclerotia were obtained. It was estimated that the total number of sclerotia in the soil comprising the root zone of the tree was more than a million. That most of the sclerotia were alive in an area that was known to have been infested for several months indicates that sclerotia under natural conditions do not soon lose their viability.

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DETERMINATION OF THE ERRORS OF ESTIMATE OF A FOREST SURVEY, WITH SPECIAL REFERENCE TO THE BOTTOM-LAND HARDWOOD FOREST REGION[1]

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INTRODUCTION

The Forest Service, under the authorization of the McSweeney-McNary Forest Research Act, is at present undertaking an exhaustive survey of the country's forest lands, by comparatively small natural or political subdivisions, such as groups of counties, with the purpose of obtaining therefrom a complete and accurate picture of the forest situation and the forest needs of the Nation. Chief among the required results of this survey are the following: Area in each type of forest cover, subdivided according to present productive condition; volume of timber by species; rate of growth on existing forests and reforesting areas; rate of forest depletion by cutting, fire, insects, and other agencies; and present national timber requirements and probable future trends.

The diversity of timber types, forest conditions, and timber growth to be encountered in such a survey, together with the scanty knowledge we now have of these in quantitative terms, make it imperative, in order to carry out the project efficiently with the funds available, that methods of gathering and compiling the enormous amount of data be carefully worked out in advance for each major forest region. This is especially important in view of the fact that the bulk of the data must be collected at first hand in the field.

Of first importance is a method of estimating the extent of data that must actually be gathered in any one large unit of area, and the nature of the survey, in order to obtain a reliable basis for estimating accurately the condition of the entire unit. The object of this paper is to present, by means of the analysis of a comparatively small set of field data gathered for this purpose from the bottom-land hardwood forest region, a practical means of determining these particulars for any given region and of making certain that the probable error of the results, for groups as small as several counties, will not exceed a previously assigned maximum.

THE GENERAL PROBLEM AND THE DATA

Fundamentally the problem is one of sampling. Its object, stated statistically, is to reduce the standard error of a mean to the practicable minimum. Theoretically this may be done either by eliminating all irrelevant variation from the standard deviation of the whole unit of data, or by increasing the number of variates or measurements, as is evident in the following formula:

$$\sigma_M = \frac{\sigma}{\sqrt{N}} \qquad (1)$$

[1] Received for publication Mar. 18, 1932; issued December, 1932.

in which

σ_M = the standard error of the mean
σ = the standard deviation of the universe, and
N = the number of variates or measurements

There are, however, practical difficulties in either of these procedures. On the one hand, the standard deviation is a characteristic of the universe dealt with. It is an aggregate of variation from an indefinite number of causes; and only that portion which is recognized by quantity and by source may be logically deducted, leaving the residual error of estimate. On the other hand, the number of variates that may be collected is limited by time and funds available for the field work of a survey. In order, therefore, to find out what reliability of the mean can be attained with given funds it is necessary to gather a certain amount of data from a given universe and, first of all, analyze the standard deviation in order to arrive at the proper value for σ, at the same time keeping cost account of the field work to establish the highest practicable numerical value of N.

With these ends in view, a preliminary survey was made in the spring of 1931 in East Carroll and West Carroll Parishes in northeastern Louisiana. These parishes lie wholly within the Mississippi River Delta region[2] and contain bottom-land hardwood forests typical of all but the southernmost portion of the Delta. The forest and nonforest types are arranged in irregular north and south belts paralleling the Mississippi River and smaller drainages. Immediately west of the Mississippi there is a belt of "batture" land that extends to the levee. It is partly to completely overflowed every year and is almost entirely forested, principally with willow (*Salix* sp.) and southern cottonwood (*Populus deltoides virginiana* (Castigl.) Sudw.). Just west of the levee is a belt of rich agricultural land, planted largely to cotton. Beyond this lie, in order, a belt of cut-over bottom-land hardwoods; a belt of agricultural land along Bayou Macon, which separates the two parishes, and on Macon Ridge; and finally a broad belt of bottom-land hardwoods which contains, in two large tracts, the bulk of the virgin timber. These so-called belts are, of course, by no means entirely of the classification given but are principally as described.

The sample survey was made by what is known as the line-plot method. Ten parallel survey lines, 3 miles apart, were run due east and west in order to cross the prevailing drainages approximately at right angles. At each 10-chain (660 feet) interval along the lines, a quarter-acre sample plot was established on which, if in the forest, measurements were made; if not, the nonforest type was noted. In this way, 240 miles of line were measured, and 1,918 plots were taken, of which 1,189 fell within the forest. Since the total area of the two parishes by the most reliable estimate is 503,000 acres, nearly 0.1 per cent of the area is actually in sample plots. Without entering into any detailed account of the manner in which the data were collected on each plot, or the exact nature of the many items of information, it is proposed to analyze the manner and extent of sampling to determine its adequacy with respect both to land area by forest and

[2] The Mississippi River Delta region extends from above Cape Girardeau, Mo., some 60 miles northwest of the mouth of the Ohio River, to the Gulf of Mexico, and comprises an area of about 29,000,000 acres.

nonforest classes and to volume of timber by species and forest-condition class.

AREA ANALYSIS

The object of the area analysis is to arrive at a correct and feasible method of estimating the land area in the following land classes, together with a statement of the error of estimate of each: Total forest land; forest land in each of 5 forest sites—ridge, flat, swamp, batture, and upland;[3] forest land in each of 7 forest conditions—virgin, culled, cut-over and restocking, cut-over and not restocking, ruined, second growth, and old field;[3] total land not in forest (nonforest); and nonforest land in each of 11 nonforest conditions.

There are two sets of data both suitable for analysis. First is the number of plots counted in each land class in terms of the entire number of plots in the survey. Analysis of these data involves homograde statistics or the statistics of attributes. The other possibility is the linear distance traversed in each land class in terms of the entire length of line in the survey. Analysis of these data involves heterograde statistics or the statistics of variables.

TABLE 1.—*Summary of land class area data for East Carroll and West Carroll Parishes, La.*

Land-area class	Line survey		Plots	
	Chains	*Per cent*	*Number*	*Per cent*
Forest site:				
Ridge	4,708	24.56	469	24.45
Flat	6,198	32.33	616	32.12
Swamp	301	1.57	23	1.20
Batture	637	3.32	65	3.39
Upland	176	.92	16	.83
Total in forest	12,020	62.70	1,189	61.99
Forest condition:				
Virgin	1,515	7.90	154	8.03
Culled	228	1.19	23	1.20
Cut-over restocking	7,654	39.93	758	39.52
Cut-over nonrestocking	1,032	5.38	93	4.85
Ruined	488	2.55	55	2.87
Old field	533	2.78	50	2.60
Second growth	570	2.97	56	2.92
Total in forest	12,020	62.70	1,189	61.99
Nonforest type:				
Cultivated farm land	4,721	24.63	470	24.51
Deadening	1,034	5.39	114	5.94
Pasture land	541	2.82	59	3.08
Wood-lot pasture	317	1.65	33	1.72
Abandoned farm land	143	.75	14	.73
Prairie				
Marsh	26	.14	3	.16
Levee	61	.32	5	.26
Roads, railroads, etc	138	.72	9	.47
Towns, villages	23	.12	2	.10
Rivers, lakes, etc	145	.76	20	1.04
Total, nonforest	7,149	37.30	729	38.01
Total area	19,169	100.00	1,918	100.00

When the data are listed according to both methods, as in Table 1, it is evident that, on the basis of percentages of area, the two methods check satisfactorily. If the resulting errors of estimate were also to check, the use of the plot count would be preferred because of the

[3] For definitions of these sites and conditions, see the following publication: LENTZ, G. H. THE FOREST SURVEY IN THE BOTTOMLAND HARDWOODS OF THE MISSISSIPPI DELTA. Jour. Forestry 29:1046-1055. 1931.

simplicity of its application, and because the linear measurement of area classes traversed in the field could then be omitted entirely.

PLOT-COUNT METHOD

The plot-count method is useful in determining whether we are dealing with a true random sample of land-area classes; that is, whether the statistical sample—the number of plots by land-area class—has been selected in such a way that any plot has been as likely of selection from the statistical universe (the bottom-land area as represented by East Carroll and West Carroll Parishes) as any other. If we have such a sample, the standard deviation of the number of plots in a given land class is

$$\sigma = \sqrt{npq} \quad (2)$$

in which

σ = the standard deviation of the number of plots
p = the chance that a plot is of a given land class
q = the chance that it is not, where $p+q=1$
n = the total number of plots

The standard error of the proportion of plots in a given land class may be written

$$\sigma_p = \frac{\sigma}{n} = \sqrt{\frac{pq}{n}} \quad (3)$$

This is a more useful measure for the final expression because it gives, directly, the standard error of a particular land class as a percentage of the total land area. For comparison with actual dispersion, however, that measure will be used which is the handier for the particular purpose. In the following application of formula 3, the cut-over restocking forest condition is used because it contains the greatest number of plots. According to Table 1 there are:

758 plots in cut-over restocking condition
1,160 plots in other conditions (both forest and nonforest)
1,918 plots in all

hence

$$p = \frac{758}{1918} = 0.395$$

$$q = \frac{1160}{1918} = 0.605$$

and from equation 2

$$\sigma = \sqrt{1918 \times 0.395 \times 0.605}$$
$$= 21.5 \text{ plots}$$

This means that, provided the conditions of random sampling have been fulfilled, it is to be expected that in 2 out of 3 additional sets of 1,918 plots the number in the cut-over restocking conditions will be within 22 of the present count.

But this is the point to be investigated. We need to know whether the standard deviation calculated in this way from the 1,918 plots is identical with the standard deviation that would be obtained if all the quarter acres of land area in the two parishes could be thrown into an urn, thoroughly mixed, and a number of samples consisting of 1,918 plots each could be drawn out. Obviously such a test can not be made. However, the principle may be tested by means of two different schemes of setting up data for the calculation of the actual standard deviation. The first of these is a system of replications or

mechanical groupings, in which, for example, group 1 is composed of plots Nos. 1, 21, 41, 61, etc., group 2 is composed of plots Nos. 2, 22, 42, 62, etc., making in all 20 groups with about 96 plots in each group. Another scheme compares the relative number of plots in cut-over restocking condition from one survey line to another, making 10 groups, one for each line.

Test by Mechanical Grouping

From the distribution of the mechanical groupings as presented in Table 2, the mean and standard deviation are reckoned as follows:

$$\text{Mean} = 37.9 \pm 0.62 \text{ plots out of } 96$$
$$\text{Standard deviation} = 2.75 \pm 0.44 \text{ plots out of } 96$$

Table 2.—*Frequency of plots in cut-over restocking condition in 20 mechanically set up groups of 96 plots each*

Group No.	Number of plots of cut-over restocking	Group No.	Number of plots of cut-over restocking
1	35	12	37
2	38	13	34
3	33	14	37
4	33	15	37
5	41	16	38
6	38	17	42
7	41	18	40
8	40	19	39
9	43	20	39
10	36		
11	37		758

Now, the standard deviation to be expected from a random sample of 96 plots is, from equation 2:

$$\sigma = \sqrt{96 \times \frac{37.9}{96} \times \frac{58.1}{96}}$$
$$= 4.79$$

It is evident that 2.75 ± 0.44 can not be accepted as an accidental deviation from 4.79. However, familiarity with the region from which these samples are drawn helps to explain how this scheme of grouping plots might result in a subnormal dispersion. Each plot is but one-eighth mile distant from its immediate neighbors on the same line. Since areas of cut-over restocking condition, when encountered in the field at all, are usually extensive, it follows that wherever one plot in this condition occurs a number of consecutive ones are likely to be found. Since each consecutive plot has been put into a consecutive group, the variability from group to group is less than the variability of random samples.

Test by Survey-Line Grouping

Table 3 gives the total number of plots on each line and the number and percentage of cut-over restocking plots on each, without regard to length of line. From these percentages the following calculations are made:

$$\text{Mean} = 39.0 \pm 2.42 \text{ per cent}$$
$$\text{Standard deviation} = 7.65 \pm 1.71 \text{ per cent}$$
$$\text{Average number of plots per line} = 192$$

TABLE 3.—*Frequency of plots in cut-over restocking condition, by survey lines*

Line No.	All plots	Frequency of cut-over restocking		Line No.	All plots	Frequency of cut-over restocking	
	Number	*Number*	*Per cent*		*Number*	*Number*	*Per cent*
1	115	46	40	7	260	118	45
2	150	53	35	8	210	96	46
3	201	71	35	9	209	117	56
4	197	72	37	10	171	57	33
5	200	71	35				
6	205	57	28	Total	1,918	758	------

However, the theoretical standard error of the proportion is, by equation 3:

$$\sigma_p = \sqrt{\frac{0.395 \times 0.605}{192}}$$

$$= 0.0353 \text{ per plot or } 3.53 \text{ per cent}$$

The abnormal variation of the areas from line to line as compared with the theoretical dispersion demonstrates that the areas of cut-over restocking lands are by no means evenly distributed over the two parishes; that there is, rather, a relationship between percentage of cut-over restocking area and "place" in the two parishes.

It may be argued that since the actual variation may be made subnormal or abnormal depending upon the method of grouping, the theoretical standard deviation of the ungrouped data—21.5 plots out of the 1,918—may be a sufficiently satisfactory one, in that it should average out the high and low dispersions. The fallacy of this will be brought out in the test problem to be discussed later.

LINEAR-MEASUREMENT METHOD

The linear-measurement method differs from the plot count in one important respect. Whereas the plot count of a land-area class is merely alternative, in that a given plot either is or is not of the class to be investigated, the linear measurement is qualitative, in that the distance traversed in the land class under investigation is measured. The problem, then, becomes one of analysis of variation of measurement on the hypothesis that the area of a given land class varies as the linear distance traversed within it. A discussion of this hypothesis will be taken up later.

To begin with, it is necessary to determine which unit length of line (e. g., one-half mile, 3 miles) gives the most stable measure of the dispersion of a land-area class. In this determination the cut-over restocking forest condition is again used.

TESTS BY CONTINUOUS UNITS

The lengths analyzed are the ½-mile, 3-mile, and 24-mile units; for each of these the standard deviation and the standard error of the mean in number of chains are calculated and reduced for ready comparison to number of chains to the half mile. (Table 4.)

TABLE 4.—*Comparison of standard deviations and standard errors of the mean for various unit lengths for survey line in cut-over restocking condition*

Length of unit	All units	Cut-over restocking statistics per one-half mile		
		Mean	Standard deviation	Standard error of the mean
	Number	*Chains*	*Chains*	*Chains*
½ mile	480	16	15.9	0.73
3 miles	80	16	8.76	.98
24 miles	10	16	4.78	1.51

The increasing standard error of the means for units greater than one-half mile in length, as shown in Table 4, clearly indicates that the variation in the data from the longer units, like the abnormal variation of the plot count when full line units were used, is correlated with "place."

TEST BY RANDOMLY GROUPED ½-MILE UNITS

Although Table 4 shows that the error of land-class estimate is less with the ½-mile unit than with the longer unit lengths, the standard deviation—15.9 chains to the half mile—is practically as great as the mean of 16 chains. This follows from the fact that the distribution is U-shaped; of the four hundred and eighty ½-mile units, 155 contain no chainage at all of cut-over restocking and 88 are entirely cut-over restocking.

On this account the standard deviation is subject to an error greater than that of unimodal distributions; and as the standard error of the mean of any distribution varies directly as the standard deviation, this also is subject to greater fluctuation in this type of distribution. Therefore, rather than to rely upon a single standard deviation from which the error of the mean is to be calculated, actual variations of means from groups or randomly selected ½-mile units were compared with the theoretical. For this purpose, the number of chains of land in cut-over restocking condition in each of the four hundred and eighty ½-mile units was tallied upon small, metal-rimmed, circular tags, such as are used for price tags. The 480 tags were thoroughly mixed in a box, drawn out one at a time, and the values noted in the order of draw. Four groupings were thus made. Their standard deviations are given in Table 5.

TABLE 5.—*Comparison of standard deviations of groups of random-selected ½-mile units in cut-over restocking condition*

Units in group	Groups	Standard deviation per one-half mile	Units in group	Groups	Standard deviation per one-half mile
Number	*Number*	*Chains*	*Number*	*Number*	*Chains*
12	40	4.71	24	20	2.61
20	24	3.82	40	12	2.72

Figure 1 summarizes graphically the work thus far on the linear-measurement method. It shows the standard deviation of ½-mile units and the standard deviation of the 3-mile and 24-mile continuous units, all from Table 4; the standard deviations of distribution of the randomly grouped ½-mile units, from Table 5; and the curve of the theoretical standard deviation of randomly grouped ½-mile units. Figure 1 indicates that unit lengths greater than one-half mile introduce systematic errors of the mean, the result of correlation of cut-over restocking areas with "place" in the two parishes. On the other hand, the actual error of the groups, after the effect of "place"

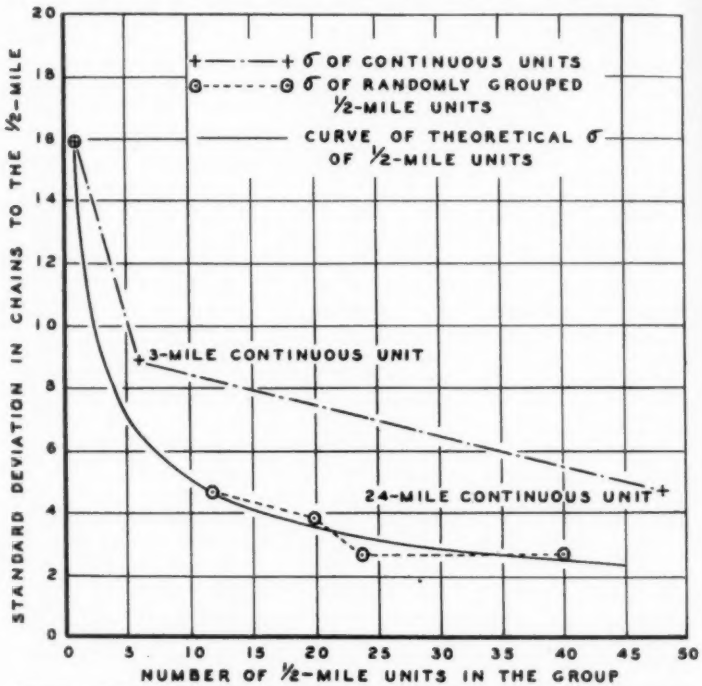

FIGURE 1.—Comparison of the standard deviations resulting from the various methods of grouping with the curve of the theoretical standard deviation. Cut-over restocking condition

is removed by random selection of ½-mile units, checks very well with the theoretical.

It is concluded, therefore, that when areas in land units similar to these two Louisiana parishes are determined by the linear-measurement method, the error of sampling of a land class may be deduced directly from the distribution of ungrouped ½-mile units.

PLOT COUNT VERSUS LINEAR MEASUREMENT

Up to this point it has been satisfactorily established (1) that the plot-count method exposes systematic variation, if present, but does not give stable errors of estimate of land-class area; and (2) that if the linear-measurement method is employed, the use of the ½-mile

unit results in errors of estimate free from systematic variation over the territory sampled. But this gives little indication of which method is to be preferred. It may be, for instance, that the error of the ungrouped plot-count data—the 21.5 plots out of the 1,918, or about 1 per cent of the land area—is sufficiently close to the truth in that it averages the subnormal and abnormal variations of the grouping schemes used. The choice of method rests upon the agreement of estimated areas with the true areas. But such comparisons can not be made for the land classes as defined because we have no true areas upon which to base them.

Recourse was had in this instance to a soil-type map of East Carroll and West Carroll Parishes upon a scale of 1 inch to the mile.[4] As the different soil types roughly parallel the Mississippi River and interior drainages just as do the forest sites and conditions, this served very well as a medium for the comparison of method, in the following manner:

The 10 parallel east and west survey lines actually determined were drawn on the map, 3 miles apart, establishing as line universes the area within 1½ miles on either side of each survey line.

The area of each soil type was planimetered by line universe. Seven soil types occurred on nine of the line universes and three soil types on the remaining one. The planimetered areas of these 66 cases were taken as the true areas of the soil types, the base for comparison of sampling method.

The sampling process on the map resembled the sampling of land-area classes in the field—(1) by counting plots at every 10 chains along the lines and noting the soil type of each, and (2) by scaling the distance in chains across each soil type traversed by the survey lines.

Test of the Plot-Count Method

The difference between the actual areas and the areas estimated by plot count were calculated in terms of the standard error of the latter for each of the 66 cases. A frequency distribution of these ratios—if the method has fulfilled the requirement of random sampling—should have a standard deviation of 1, and its departure from 1 may be considered a measure of the failure of the sampling method. The following example of Sharkey clay soil type on line universe 1 serves as an illustration:

Planimetered area of Sharkey clay = 3,456 acres
Planimetered area of line universe = 26,132 acres

$$\text{Relative area of Sharkey clay} = \frac{3456}{26132} = 0.132$$

Number of Sharkey clay plots on line 1 = 18
Total number of plots on line 1 = 112

$$\text{Relative number of Sharkey clay plots} = \frac{18}{112} = 0.161$$

Standard error of relative number of Sharkey clay plots:

$$\sigma_p = \sqrt{\frac{0.161 \times 0.839}{112}} = 0.035$$

[4] Worthen, E. L., and Belden, H. L. Soil survey of East Carroll and West Carroll Parishes, Louisiana. U. S. Dept. Agr., Bur. Soils Field Oper. 1908, Rpt. 10: 875-898, illus. 1911.

Difference between actual and estimated areas in terms of the standard error of the latter is

$$\frac{0.132-0.161}{0.035}=-0.8\sigma$$

The distribution of the ratios is shown in Table 6.

TABLE 6.—*Distribution of the ratios of actual errors of soil-type area to estimated errors by the plot-count method*

Error [a]	Frequency	Error [a]	Frequency	Error [a]	Frequency	Error [a]	Frequency
+5.5		+2.0	6	−1.5	5	−4.5	
+5.0	1	+1.5	5	−2.0	2	−5.0	
+4.5	1	+1.0	6	−2.5	1	−5.5	1
+4.0		+0.5	6	−3.0	2		
+3.5		0	8	−3.5			66
+3.0	3	−0.5	11	−4.0			
+2.5	1	−1.0	7				

[a] The difference between the actual area and the estimated area in terms of the standard error of the estimated area.

Their standard deviation is

$$\sigma=1.72\pm0.149$$

Had we assumed the sampling method to have fulfilled the requirements of random sampling we would have taken for granted that the standard error of the plot count would give the range within which the true error lies two times out of three, or with odds of 2 to 1. We find, however, that the actual error is, most probably, 72 per cent higher; that the estimated standard error is in reality $\frac{1}{1.72}$, which, by reference to the normal curve of error, is equivalent to odds of but 0.8 to 1.

TEST OF THE LINEAR-MEASUREMENT METHOD

In testing the linear-measurement method essentially the same kind of comparison was made as in the case of the plot-count method. The following is an illustration of the calculation in one case, employing the Sharkey clay on line universe 1:

Relative area of Sharkey clay = 0.132 (as above).

Statistics from frequency distribution of linear measurements, shown in Table 7:

TABLE 7.—*Frequency distribution of scaled distances of Sharkey clay land on line 1*

Chains of Sharkey clay to the one-half mile	½-mile units	Chains of Sharkey clay to the one-half mile	½-mile units	Chains of Sharkey clay to the one-half mile	½-mile units	Chains of Sharkey clay to the one-half mile	½-mile units
	Number		*Number*		*Number*		*Number*
0	23	15		30		40	3
5		20	2	35			
10	1	25				Total	29

Mean = 5.17 chains to the half mile
Standard deviation = 10.7 chains to the half mile
Standard error of mean = 1.99 chains to the half mile

Hence, relative area of Sharkey clay $= \frac{5.17}{40} = 0.129$

Standard error or relative area of Sharkey clay:

$$\sigma_p = \frac{1.99}{40} = 0.050$$

Difference between actual and estimated areas in terms of the standard error of the latter:

$$\frac{0.136 - 0.129}{0.050} = +0.1\sigma$$

The distribution of the ratios are shown in Table 8.

TABLE 8.—*Distribution of the ratios of actual errors of soil-type area to estimated errors by the linear-measurement method*

Error [a]	Frequency	Error [a]	Frequency	Error [a]	Frequency	Error [a]	Frequency
+4.2	1	+2.1	-------	0	9	−2.1	-------
+3.9		+1.8	3	− .3	15	−2.4	-------
+3.6		+1.5	1	− .6	4	−2.7	-------
+3.3	1	+1.2	2	− .9	2	−3.0	1
+3.0	-------	+ .9	7	−1.2	4		-------
+2.7	-------	+ .6	8	−1.5	-------		66
+2.4	-------	+ .3	7	−1.8	1		

[a] The difference between the actual area and the estimated area in terms of the standard error of the estimated area.

The standard deviation is:

$$\sigma = 1.05 \pm 0.093$$

This does not differ significantly from 1 and therefore satisfies the requirement that the estimated errors be the true errors of estimate. Furthermore, since no correlation is found between the ratios and the size of area estimated, it is proved that the area of a land class, in a universe represented by these two parishes, varies as the linear distance traversed within it.[5]

COMPARISON OF METHODS

The final results of the two sampling methods are compared in Table 9, the last three columns of which show the errors of estimate. On the assumption that plot counts at spacings of 10 chains by 3 miles satisfy the conditions of random sampling, the resultant error by this method is the lesser. As has been brought out, however, this assumption is erroneous, as it requires, for the soil-type data at hand, a correction factor of 1.72. This correction has been applied in the table, the two right-hand columns bringing out the properly comparable errors.

[5] At first thought it seemed more logical to one of the authors that the area of a land class should vary as the sums of the squares of the linear distances traversed within it. Walter H. Meyer, of the Pacific Northwest Forest Experiment Station, tested this hypothesis and found that it leads to estimates that are too high whenever a single land class occurs as an extensive, unbroken area traversed by two or more survey lines.

TABLE 9.—*Relative areas of soil types and comparison of their standard errors of estimate by different sampling methods*

Soil type [a]	Relative areas of soil types			Standard error of area as a percentage of corresponding area		
				By plot count		By linear measurement
	By planimeter	By plot count	By linear measurement	Random sampling	Corrected for sampling [b]	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
YS	5.5	5.3	4.2	10.0	17.2	24.0
YL	6.4	5.8	6.5	9.5	16.3	15.0
WC	17.2	19.6	18.1	4.7	8.1	9.3
SC	25.6	24.2	24.5	4.1	7.1	7.9
WSL	8.9	9.8	9.9	7.1	12.2	14.1
RL	17.8	17.3	18.7	5.1	8.8	9.0
CS	18.3	18.0	16.6	5.0	8.6	9.8
Total	[c] 99.7	100.0	[d] 98.5			

[a] Symbols for soil types are those given on the map mentioned in text.
[b] This correction is 1.72 times the value obtained on hypothesis of random sampling.
[c] Three-tenths of 1 per cent of the total area is made up of 3 soil types which are not included because they are not encountered in the plot-count method.
[d] The 3 soil types not given in the table account for the remaining 1.5 per cent.

In six of the seven soil types, the corrected error of the plot-count method is still less than the error of the linear-measurement method. It is very doubtful, however, whether the differences in its favor are to be considered as a recommendation, for the correction is an empirical one, and may safely be applied only to this soil-type test. The error of linear measurement, on the other hand, is the outcome of variation actually encountered; it calls for no correction, because by comparison with the universe from which the data were drawn, it satisfies the limitations of random sampling.

VOLUME ANALYSIS

The volume data consist of the board-foot contents, by species, of the timber on 1,208 sample plots [6] taken in the forest. From these the following values for all species together have been calculated:

Mean = 659 board feet
Standard deviation = 854 board feet
Standard error of mean = 24.5 board feet

But the standard error of the mean varies directly as the standard deviation of the distribution, and the latter measure is an aggregate of variation from many causes. One of these readily recognized in the field is the forest condition.

The plots were accordingly sorted by condition. The mean volume and standard deviation of each are as given in Table 10. The standard deviations are, in fact, standard errors of estimate of plot volume free from the influence of forest condition.

[6] This does not agree with the 1,189 forest plots of Table 1. The field instructions called for the measurement of a forest plot whenever a nonforest plot happened to fall within 2 chains of the forest, the purpose being simply to strengthen volume data. The added 19 plots were not used in area analyses.

TABLE 10.—*Means and standard deviations of plot volumes by forest condition*

Forest condition	Basis, plots	Mean volume per plot	Standard deviation of volume per plot
	Number	*Board feet*	*Board feet*
Virgin	155	1,886	1,232
Culled	22	1,779	792
Cut-over restocking	761	478	533
Cut-over not restocking	100	354	576
Ruined	50	96	200
Old field	55	360	450
Second growth	56	700	976

The average of these, weighted by number of plots, may be expressed:

$$\bar{\sigma} = \sqrt{\frac{N_1\sigma_1^2 + N_2\sigma_2^2 + \ldots\ldots\ldots + N_n\sigma_n^2}{N_1 + N_2 + \ldots\ldots\ldots + N_n}} \quad \text{------ (4)}$$

in which

$\bar{\sigma}$ = the weighted mean value of standard deviations from n frequency distributions

σ = the standard deviation of each distribution

N = the number of plots in each distribution

and subscripts 1, 2, ... n refer to the individual distributions.

The weighted mean value of the standard deviations of Table 10 thus becomes 684 board feet.[7] Hence, by equation 1, the standard error of the mean, free from the influence of forest condition, becomes 19.7 board feet.

The data were further scrutinized for evidence of relationship between the remaining variation of the mean and other factors, particularly site and locality, but nothing of significance was found.[8] Hence it is concluded that 19.7 board feet to the quarter acre is the error of sampling. This is 2.99 per cent of the mean volume.

The error of the total volume of the two parishes, however, must be greater than 2.99 per cent because it is affected not only by volume variation from plot to plot, but also by the error of estimate of total forest area. In short, the correct estimate of volume on a large forest area is

$$(A \pm \sigma_A)\,(M \pm \sigma_M)$$

in which

A = the total forest area, in this case in quarter acres

M = the mean volume to the quarter acre

σ_A = the standard error of the total forest area

σ_M = the standard error of the mean volume

This equation has been shown to be:

$$AM \pm \sqrt{A^2\sigma_M^2 + M^2\sigma_A^2} \quad \text{------ (5)}$$

[7] We may calculate a measure of the effect of forest condition upon the volume of hardwoods in the two parishes. For the squared variation of volume free from the influence of condition, [=(684)²]+the squared variation of volume associated with condition=the total squared variation [=(854)²]. From this equality we find that 35.8 per cent of the total squared variation of volume is associated with forest condition. Hence the correlation ratio, which is calculated directly from the square root of this value, is 0.598 ±0.018.

[8] A standard deviation below the 684 board feet obtained by stratifying according to condition may be arrived at by grouping the plots either systematically or at random and finding the standard deviation of the means of the groups. This value times the square root of the number of plots per group gives the residual standard deviation of plot distribution. With 20 or less groups a standard deviation of 566 board feet has been obtained. However, there is no justification for the reduction as it is impossible to state just what factor or factors of correlated variation have been averaged out in the process of grouping.

The best estimate of the total area of the two parishes is 503,000 acres,[9] and this is taken as without error. The total forest area, calculated by the linear-measurement method, is 62.7 ± 2.95 per cent of this, or $1{,}261{,}524 \pm 37{,}215$ quarter acres. Since the mean volume to the quarter acre is 659 ± 19.7 board feet, equation 5 gives a total volume in the two parishes of 831 ± 34.9 million board feet and the standard error is 4.20 per cent of the total.

We now have a working basis for calculating the total number of miles of survey line that must be run across land units similar to East Carroll and West Carroll Parishes in order to determine the total volume of merchantable timber within any given limit of accuracy. The total number of miles, with a plot taken every 10 chains, is one-eighth of the total number of plots and, in the present case, almost exactly one-fifth of the number of forest plots (for forest area equals 62.7 per cent of the total area). From equation 1 we have

$$N = \left(\frac{\sigma}{\sigma_M}\right)^2 \qquad (6)$$

where

N = the number of forest plots

The numerator to use for ready calculation of equation 6 should be that standard deviation which measures variation of plot volume and forest area, but which is free from the influence of variation in forest condition. We have this measure, but it is expressed as the standard error of the mean (or total)—the 4.20 per cent arrived at above. The corresponding standard deviation is $\sqrt{1{,}208}$ plots times this value. The denominator should be the standard error expressed as a percentage of the mean (or total)—that is, the measure of precision desired.

The results in number of plots or number of miles of line must be coordinated with the results similarly obtained from other analyses, such as volume of certain important species or volume by log scale, before any final estimate of the amount of data needed can be rendered.

COORDINATION AND APPLICATION OF ANALYSES

In conclusion, it remains only to show the practical use or application of the calculated errors of sampling and the way in which the errors for the different items of area and volume are coordinated. The vital question, indicating the raison d'être for the foregoing analyses, is: How many miles of survey lines must be run to obtain acceptable limits of error in all important phases of the survey? From the number of miles, the distance between lines is of course readily calculated when the area of the land unit to be surveyed is approximately known.

To answer this question, it is necessary first to coordinate the different errors for the same mileage of line by putting them in such form that they can readily be compared. This is conveniently done by making a table that shows the standard error (in percentage of

[9] This figure has been arrived at after comparing estimates of the area concerned as established by the Bureau of Chemistry and Soils and the Bureau of Agricultural Economics, United States Department of Agriculture, and the county surveyor.

the mean) for each important item for several selected mileages of line. The mileages selected should be within the range of practical possibility as determined by the size of the land unit to be surveyed, the cost per mile of line, the funds available, the approximate permissible errors, etc. Table 11 is a sample portion of such a tabulation.

TABLE 11.—*Standard errors of important items for different total lengths of line*

[A sample portion of a tabulation from which the required mileage of survey lines can most easily be determined. Eight plots taken per mile. Each standard error expressed as a percentage of the mean of the item concerned]

Item to be evaluated	Percentage of the mean for the indicated total number of miles of line run				
	200	300	500	1,000	1,200
	At standard error of—				
	$x\sqrt{5}$	$x\sqrt{3.33}$	$x\sqrt{2}$	x	$\frac{x}{\sqrt{1.2}}$
Area:					
Virgin forest	15.4	12.6	9.8	6.9	6.3
Cut-over-restocking forest	4.7	3.8	3.0	2.1	1.9
Total forest	3.2	2.6	2.0	1.4	1.3
Volume (including error of area):					
Virgin forest	16.6	13.5	10.5	7.4	6.7
Cut-over-restocking forest	6.5	5.3	4.1	2.9	2.6
Red gum	12.3	10.1	7.8	5.5	5.0
Total forest	4.5	3.6	2.8	2.0	1.8
(Etc.)					

The greatest acceptable error in the most important item (in this case, and usually, total merchantable volume) may then be decided upon and the corresponding mileage read directly or interpolated from the table. The errors in other items for this mileage are then noted and if any of them are unsatisfactory the mileage may have to be increased. Many compromises and concessions will invariably be necessary before the errors in most or all of the items are declared satisfactory for a financially practicable mileage.

In the bottom-land hardwood region the forest survey proper will be started in a land unit of about 5,500 square miles in northern Louisiana. After a detailed study of a tabulation of expected errors for given mileages, of which Table 11 is a small part, it is estimated that the results for this unit will be sufficiently close to the truth if the total mileage run is 550. This gives a line interval of 10 miles. The standard errors expected for the several items of Table 11 are about 95 per cent of those tabulated therein in the 500-mile column.

SUMMARY

Samples of land-area measurements and timber-volume data were taken in two parishes in northeastern Louisiana by the line-plot system, for the purpose of determining the probable errors of estimate by land-area classifications and timber volumes to be expected from a projected forest survey of similar nature of 29,000,000 acres in the bottom-land forest region.

The land-area analyses disclosed that the number of plots in the various land classes, while correctly establishing their proportional areas, can not be considered as random samples of the land classes.

This is because neither the standard deviation as calculated from 20 mechanically set-up groups nor the standard deviation as calculated from the variation between the survey lines, checked with the standard deviation to be expected upon the hypothesis of random sampling.

The linear-measurement method of area by land classification was analyzed as an alternative to the plot-count method. The error of estimate was tested by using unit lengths of one-half mile, 3 miles, and 24 miles, and was found to increase with unit lengths longer than the one-half mile.

The comparison of the errors of estimate as between the plot-count and the linear-measurement methods was based upon a test problem from the soil-type map of the two parishes. The true areas in each soil type were arrived at by planimetering the map. The soil types were then sampled on the map in a manner analogous to that by which land-area classes were sampled in the field. The comparison of sampling method is based upon the agreement of the actual with the estimated sampling errors. It was found that the actual errors of the linear-measurement method agreed with the estimated errors.

In the volume analyses, the standard error of the mean volume in board feet was found to be about 3 per cent of the mean volume per ¼-acre plot; and this value is the residual after taking out that portion of the error which is due to variation in forest-condition classes. Since the total volume in the forests of the two parishes is the product of the mean volume per ¼-acre plot and the number of quarter acres in forest area, with their respective errors of estimate, the standard error of estimate of the total volume becomes slightly higher than 4 per cent of the total volume.

The results of the area and volume analyses were used as the basis for the recommendation that in the forest survey proper the line interval be 10 miles.

DEFINITIONS OF HONEY COLOR GRADES [1]

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INTRODUCTION

Grading according to color has long been an important factor in the marketing of honey. In different countries and by different organizations of beekeepers and honey dealers, various methods have been employed for evaluating and describing the colors of honey. In some instances names of color grades have been used without any attempt to define the grades or to employ standard colors for grading. In other cases colored glasses or bottles of colored liquids (often quite different in color from honeys) have been used. Obviously it is desirable that some accurate method be devised for defining and measuring the color grades of honey and that the same standards be widely used.

IMPORTANCE OF COLOR GRADING

The most important reason for grading honey by color is that flavors of honeys from any given plant source vary with color, as certain examples will show. In the irrigated regions of the western part of the United States alfalfa honey is produced, usually with an admixture of honey from sweetclover but with less than the customary admixtures of nectars from other plant sources. In those parts of the irrigated region in which the altitude is high the honey from alfalfa and sweetclover is the most delicate and mild obtainable from these plants. In the Imperial Valley, lying below sea level, the honey from these plants is a rather dark amber, and the flavor is stronger and less delicate. It was formerly supposed that these differences were caused by admixtures of other nectars, but it is now known that they are due to the effect of climate, soil, and altitude on the color of the nectar of the plants. If, therefore, a buyer of honey desires either strong or mild alfalfa-sweetclover honey, it is most convenient to designate the color desired, since flavor varies with color.

Similar differences are found in many other honeys. Honey from white and alsike clovers varies from Water White to rather Dark (reddish) Amber, and the flavors differ much as do those of alfalfa-sweetclover honeys, the lighter honeys having the more delicate flavor. For any given plant source, the lightest and mildest honeys usually come from areas near the northern limits of the secretion of nectar from that plant.

Received for publication Jan. 15, 1932; issued December, 1932. The work here reported was carried out during the summers of 1922, 1923, and 1924, while the author was connected with the Bureau of Entomology, U. S. Department of Agriculture, and determinations of light transmissions were made at that time. Later computations were made at the New York State College of Agriculture. A considerable number of persons assisted in the early work. The author is responsible for the final calculations, for the conclusions reached, and for the recommendations of definitions for the honey color grades. The Bureaus of Entomology and Agricultural Economics are responsible for the grades recommended in earlier publications, in the devising of which the author took no part.

With certain exceptions mentioned later, it may be said that in general for any given plant origin of honey, the lighter the honey the more delicate the flavor. It thus becomes important to designate the color of honey, for the color designation is of more value in indicating flavor than the name of the plant or plants from which the nectar is derived. Since there is no way of describing flavors as such, some progress at least will be made toward classification by flavors when accurate color grading is made possible. Color grading is, therefore, not a subordination of flavors but is the most accurate way of designating flavors thus far devised.

There are certain honeys for which the demands of the market can not be met on the basis of color grading. Chief among these in the United States is buckwheat (*Fagopyrum esculentum*) honey. This honey is dark and strong in flavor. Within the limits of the United States there is considerable variation in buckwheat honeys. In southern New York and northern Pennsylvania, buckwheat honey is opaque and dark with a slightly purple cast, while in certain areas of the Mid-West this honey is more transparent and is dark amber in color, red and yellow tones predominating. There is a heavy demand for buckwheat honey by the Jewish trade, chiefly in the eastern cities, and for export; for both trades the demand is for the opaque, dark, purplish honey. It is also required that the honey be granulated with a fine grain, even though for certain purposes for which this honey is used, it is liquefied. The flavors of different buckwheat honeys vary, although all of them are strong; it is the flavor of the opaque eastern honey that is preferred in the markets mentioned. Obviously it is impossible to give a color designation that will properly grade buckwheat honey, especially since the honey must be granulated to meet the demands of the market.

Similar conditions seem to prevail with the heather honey of Europe and perhaps also with the honeydew honeys of central Europe. However, the fact that color grading is not feasible in all instances does not make it any less valuable for grading the majority of honeys.

PREVIOUS WORK

The United States Department of Agriculture has indorsed a color grader for honey (Sechrist [2] and Sechrist and Samson [3]) which is used in grading all honeys intended for export and to some extent those intended for domestic markets. While this grader has largely superseded others and has brought about greater uniformity in color grading, the instrument is so expensive that it is impractical for most beekeepers to buy it.

Any grading standard should be of such a nature that the different grades can be defined in words and figures. It should not depend on comparisons with some manufactured article, on the use of a piece of apparatus manufactured by one firm, or on the experience and skill

[2] SECHRIST, E. L. THE COLOR GRADING OF HONEY. U. S. Dept. Agr. Circ. 364, 8 p., illus. 1925.

[3] ——— and SAMSON, H. W. UNITED STATES STANDARDS FOR HONEY RECOMMENDED BY THE UNITED STATES DEPARTMENT OF AGRICULTURE... U. S. Dept. Agr. Circ. 410, 32 p., illus. 1927.

——— and SAMSON, H. W. UNITED STATES GRADES, COLOR STANDARDS, AND PACKING REQUIREMENTS FOR HONEY. U. S. Dept. Agr. Circ. 24, 32 p., illus. 1927.

of a limited number of individuals. The standard honey grader indorsed by the Department of Agriculture is not based on suitable definitions. For the present deficiencies of definition the writer is chiefly responsible, as appears from the following paragraphs. In the published discussion of this grader [4] the percentages of lights of various colors transmitted through 1 cm of honey are given for the darker limits of the several color grades established, from which it might be assumed that these color grades are adequately defined. Such, however, is not the case, for (1) the figures published fail to correspond with the light transmissions measured for the honeys on the darker limits of the several grades, and (2) the official instrument is not based on the published figures.

The foregoing criticism of the definitions of the Department of Agriculture grades calls for some explanation. Before leaving the service of the Bureau of Entomology, the present writer undertook, with the help of his associates in this work, to draw up a table giving the approximate lower limits of light transmissions of three selected wave lengths, as a guide in the establishment of color grades. At the time this table was made it was thought that the limits indicated were fairly satisfactory, and at least moderately accurate. Accordingly, when Sechrist and Samson prepared their circular, they included this table without change except for the omission of figures for two secondary grades. A reexamination of the data for the various honeys studied now discloses the fact that these figures are entirely unsatisfactory, and as definitions for the darker limits of the several grades are practically valueless.

The inadequacy of these figures is indicated by the data shown in Table 1. This table gives the minimum transmission of light for each wave length just as it was prepared by the author and published in part by Sechrist and Samson. After each of these supposedly definitive figures is shown the number of instances among the 290 honeys studied in which the light transmission for the wave length in question is lower than the minimum set by the definition. The total of such cases (two or even more may occur in a single honey) is 269, which is entirely too high a percentage of error to give any validity to the assumed minimum limits. In general, the trends are such as are found in honeys of the several grades, but the actual figures are inaccurate for the purpose for which they were used.[5] It is therefore clear that so far there are no satisfactory definitions of color grades for honeys. The purpose of this paper is to propose such definitions, based on measurements of light transmission through honeys of a wide variety of colors. Aside from the one attempt mentioned, there appears to be no previous publication that defines color grades for honey.

[4] —— and SAMSON, H. W. Op. cit. (Circ. 24, p. 29).

[5] The author regrets the necessity of pointing out errors in papers by his former colleagues, but since he is chiefly responsible for the original errors, the blame may be placed where it belongs. Sechrist and Samson are in no way responsible for these figures.

TABLE 1.—*Examination of definitions of honey color grades adopted by the United States Department of Agriculture on a basis of minimum transmission of blue, yellow, and red light, showing the number of cases in which the definitions are defective in the 290 honeys studied*

Grade	Blue light		Yellow-green light		Red light	
	Minimum light transmission in definition	Samples below minimum	Minimum light transmission in definition	Samples below minimum	Minimum light transmission in definition	Samples below minimum
	Per cent	*Number*	*Per cent*	*Number*	*Per cent*	*Number*
Water White	64	8	77	14	81.5	13
Extra White	60	9	76	12	80.5	11
White	50	19	71	20	78	21
Extra Light Amber	48	4	70	4	77	4
Light Amber	34.5	15	63	23	73	23
Extra Amber [a]	29		60.5		70.5	
Amber	15	17	49	22	63	30
Dark Amber [a]	6		43		59	

[a] The grades Extra Amber and Dark Amber are not included in the U. S. Dept. Agr. Circ. 24, table. p. 29. Exceptions to definitions for these grades must here be included in the primary grades, since the extra grades are not clearly indicated in the series of 290 honeys.

MATERIAL AND METHODS

The determinations of light transmission through honeys, here recorded were made during the summer of 1922. Previous to and during that summer, beekeepers in all parts of the country were requested to send samples of honey for this study, and each beekeeper was asked to state the chief nectar source or sources from which the honey was derived. Four hundred and fifty-five samples were received and tasted in the laboratory, and the opinions of the producers regarding the sources of the honeys were found to be quite accurate. From all the honeys received, 290 were finally selected for detailed study.

The honeys selected ranged from the lightest produced in the United States to those so dark that practically no light passed through a stratum 1 cm thick. Most of the honeys were of floral origin, but a few of the darker samples doubtless contained some honeydew honey.

In the measurement of light absorption by liquids, several methods are available. One commonly used is to determine the extinction coefficient; that is, the reciprocal of the depth of solution required to reduce the intensity of light of a given wave length to a definite percentage of that of the entering light. Another method sometimes employed is to measure the energy of the light passing through a liquid of definite thickness and compare it with the energy of the light entering it. Measurements of this type were made by Kutzner [6] for a few German honeys, but this method, while perhaps the most exact, presents difficulties and unnecessary complications. The method employed in the present work was to measure, by means of a spectrophotometer, the percentage of light of selected wave lengths passing through a stratum of honey 1 cm thick.

Kutzner claims that this method is accurate only to within 3 or 4 per cent, as compared with the light-energy method, but there are other complications, such as turbidity, which complicate all measure-

[6] KUTZNER, W. ZUR PHYSIK DER HONIGFARBEN. Arch. Bienenk. 9: 185–195, illus. 1928.

ments and which appear to make the method adopted for this work as good as could be chosen. Perhaps the greatest source of error arises from the fact that when the percentage of light passing through a 1-cm stratum of honey is determined by means of the spectrophotometer, the human eye is used to make comparisons of intensities, and this introduces an error which sometimes may be considerable. While determinations were made as accurately as possible, it is useless to assume extreme accuracy, and no such accuracy is necessary, for the honeys of the selected series could often be transposed without material difference in the appearance of the series.

In the early stages of the work, measurements of light transmissions were made for a considerable number of wave lengths, but it was found that there are no regions of the visible spectrum in which unusual absorption occurs (absorption bands), and that when a large number of determinations are made, they form a smooth curve. For practical purposes, therefore, it was found to be entirely satisfactory to confine the routine determinations to three wave lengths, namely, 480, 580, and 680 mμ, these wave lengths being those of the colors blue, yellow-green, and red.[7]

Since the chief purpose of this work was to establish definable color grades for honey, the selected honeys were arranged in series according to their appearance to the eye, from the lightest to the darkest. All the honeys were carefully liquefied to prevent differences due to the deflection of light by included crystals, care being taken not to darken the honeys by overheating.

It is probably true that the proportion of honeys of different color grades in this series, as finally adopted, does not represent the proportion of these grades as they occur in the markets, but such agreement is not necessary. The samples were obtained from all parts of the United States and a majority of them are honeys found in the main honey markets. In the lighter ranges of the series, the gradations were so fine that usually any two adjacent samples might have been transposed without the change being noticed, but in the darker honeys such a transposition would usually have been easily detectable. While the gradations are finer in these honeys, this is permissible because it is chiefly in the lighter honeys that the closer color distinctions are now demanded in the trade.[8] It should also be noted that in the darker honeys of the series the gradations are less clear and the measurements of light transmissions are more erratic. This was due at least in part to the fact that turbidity is usually more pronounced in darker honeys, but it may also have been partly due to the fact that the darker end of the series was less complete than the lighter.

After the honeys had been arranged in order, some of the most experienced honey graders in the country were asked to indicate where in the series the several color grades should begin and end. Everyone found this a difficult matter to determine, especially in the

[7] The measurments of light transmission were made by Bernard Kurrelmeyer, who worked on this problem for two summers. The third summer the work was continued by E. W. Tschudi. Throughout all this work, Prof. A. H. Pfund, of the Johns Hopkins University, freely gave advice and assistance. The arrangement of the 290 honeys in color series was the result of cooperation of the entire staff of the bee culture laboratory, as well as of several experienced honey dealers.

[8] On a uniformly ascending scale of color in the Department of Agriculture honey color grader, the total length of the scale being 14 cm, the following distances are assigned to the various color grades: Water White, 0.8 cm; Extra White, 0.9; White, 1.7; Extra Light Amber, 1.6; Light Amber, 3.5; Amber, 2.9; Dark, 2.6. There are many honeys darker than the darkest part of this scale, so that the actual range of dark honeys is much greater than here indicated. The inequality in length of the different grades is explained by the need for closer color distinctions in the lighter honeys.

lighter honeys. In order that personal prejudice might play no part in the decisions, the floral sources of the honeys were not revealed while the judgments were being passed. To obtain different sets of opinions, the honeys were exhibited at the February, 1923, meeting of the American Honey Producers' League and later at a meeting of beekeepers in Wisconsin. At the meeting of the American Honey Producers' League, a decision as to the limits of the several grades was reached by practically unanimous agreement, separate opinions being taken of a selected set of experienced honey dealers and of all other beekeepers in attendance. These decisions of course referred only to numbers in the exhibited series and had no relation to the actual definitions of the grades.

Previous to this work, different undefined names had been used in the trade for color grades of honeys. The question as to the best designations for the different color grades was discussed at the meeting of the American Honey Producers' League in 1923 and it was decided to retain the names in most common use for the primary color grades.

During this work certain beekeepers insisted that the color grades in common use, especially those for the lighter honeys, were too comprehensive, and that finer subdivisions would be helpful. It appears to be a common experience in the color grading of commodities that with continued use finer and finer subdivisions are demanded. In establishing a grading system, it therefore seemed desirable to make provision for later subdivisions as they might be needed. For such subdivisions of the primary color grades, the additional word "extra" was suggested (except for the lighter subdivision of the dark grade, which is Dark Amber), it being the intention to include in the extra grade about 30 per cent of the lightest part of the primary grade. Thus the grade Extra Light Amber would include about 30 per cent of the lightest honeys of the primary grade Light Amber. The opinion of the honey handlers present at the meeting of the American Honey Producers' League was asked regarding the limits of these, extra grades as well as of the primary grades. The proposed limits were so favorably received that all of them were adopted, except those for the Extra Light Amber grade, which were later changed.

It should be pointed out that the color grades here proposed, or any that might be adopted, are wholly arbitrary, for there are no natural color groupings in honey. Even for honeys of practically pure floral origin, no natural color grouping can be detected, and, furthermore, honeys are so blended in the hive or during extraction that even if natural color groupings occurred in honeys of pure floral origin, the boundaries would be obliterated in the blends. As a result of these facts, there are often border-line cases in which it is impossible to assign a honey definitely to a certain color grade, but in the usual handling of honeys on the market, these cases are less frequent than might be anticipated.

THE ABSORPTION AND DISPERSAL OF LIGHT BY HONEY

When light passes through honey to the eye, the impression formed through reception of the light stimulus is produced by light of various wave lengths. When white light strikes one side of a jar of honey and penetrates the honey, a considerable proportion of the light is absorbed, but this absorption is not equal for lights of all wave lengths.

The absorbed light does not reach the eye but is changed to some other form of energy, chiefly heat. The light which leaves the honey and reaches the eye is therefore a mixture of lights of various wave lengths, in proportions different from that in which they occur in white light, hence the honey appears to be colored.

If honey were clear, more light would pass through than is usually the case, but honeys contain myriads of minute particles of various sizes which either absorb light or deflect the light rays from their direct course. These particles include masses as large as pollen grains, of which there may be thousands in a pound of honey, or other foreign particles which are accidentally included in honeys. The major part of the deflection of light is caused by the presence in all honeys of matter in a colloidal state. The composition of these honey colloids is not altogether understood, but probably they are chiefly dextrins. Honeys differ widely in the turbidity caused by such colloids, some honeys appearing quite clear and others decidedly turbid. The proportion of light which passes through honey is, then, determined not only by the light-absorbing power of the liquid constituents of honey but also by the suspensions occurring in it. The turbidity of honeys seriously complicates the study of light transmission, and gives rise to difficulty is assigning minimum light transmissions for honeys of the several color grades.

The absorption of light varies of course with the thickness of the jar containing the honey, as does also light dispersion or turbidity. Even a Water White honey shows a distinctly amber color if the jar is of considerable thickness, and it also appears more turbid.

The chief constituents of honey are water and several sugars. As light passes through a solution of such sugars, there is some absorption, but this is negligible so far as giving color to the honeys is concerned. Practically all the liquid ingredients of honeys are colorless, by which is meant that they absorb little or no light and the light absorbed is practically the same for all wave lengths. But in addition to the chief ingredients of honey, there are derived through the nectars certain plant dyes which do not permit light to pass without exhibiting differential light absorption. Since honeys from different plant sources differ in color, and since under varying conditions of climate and soils the honey from any one plant source may vary widely in color, it is clear that the colors of honey are due to materials of plant origin, derived through the nectars.

MEASUREMENT OF LIGHT TRANSMISSION

After the honeys had been arranged in series according to color, the proportion of light of each of the three selected wave lengths that passed through 1 cm of each of the 290 honeys was measured. In case any honey had begun to show crystal formation before the light transmissions were determined, it was again carefully liquefied.

It is not necessary to record all the measurements made on these honeys, but a few examples will be helpful in an understanding of the difficulties arising from an attempt to correlate light transmissions with the colors as detected by the eye. The lightest honey in the series, as determined by the eye, permitted 77.5 per cent of the blue light (480 mμ) to pass through, 85 per cent of the yellow-green light (580 mμ), and 91 per cent of the red light (680 mμ). This was a

honey from black sage (*Romona stachyoides*). These percentages are not, however, the highest for any of the three wave lengths as measured by the spectrophotometer, for an alfalfa honey in the series permitted 80 per cent of the blue light and 96 per cent of the red light to pass. Turbidity in the sage honey caused more of the light to be dispersed, although the sage honey appeared lighter to the eye.

A white-clover honey, classified as White, permitted the following proportions of the light to pass: 480 mμ, 58 per cent; 580 mμ, 74 per cent; 680 mμ, 79 per cent. A comparison of the lightest honey with this white honey from clover shows that the reductions in light transmission are relatively rapid. The darkest honey of the series, of unknown floral origin but probably containing some honeydew honey, allowed the following percentages of light to pass; 480 mμ, 0 per cent; 580 mμ, 0.5 per cent; 680mμ, 15 per cent.

After the honeys had all been measured for the transmission of the lights of the three wave lengths, it was found that the percentages of transmitted light did not fall into a series comparable to that made according to the color appearance of the honeys. This variation is shown in the data from the first 10 lightest honeys of the series. (Table 2.) All 10 of these honeys were so nearly alike in color that it was difficult to tell one from the other. If the data of the light transmissions of these 10 honeys are plotted as curves, a wide band is formed, for the spread is over a range of about 20 per cent.

TABLE 2.—*Percentage of light of different wave lengths passing through the 10 lightest honeys studied*

Honey No.	Blue light (480 mμ)	Yellow green light (580 mμ)	Red light (680 mμ)	Floral source
58	77.5	85.0	91	Black sage (*Ramona stachyoides*).
101	75.0	81.5	87	Sage (*Ramona* spp.).
176	70.0	80.0	85	Fireweed (*Epilobium angustifolium*).
102	67.0	76.0	85	Do.
305	63.0	74.0	82	Do.
306	61.0	74.0	79	Milkweed (*Asclepias* sp.)
197	80.0	92.5	96	Alfalfa (*Medicago sativa*).
196	70.5	80.0	91	Do.
174	75.0	84.0	89	Do.
37	64.0	74.0	75	Button sage (*Ramona* sp.).

In order to show the extreme limits of light transmission that may fall within a single market grade, the following data are given from the honeys that fall within the lighest classification, known as Water White. For blue light (480 mμ) the highest transmission was 80 per cent, the lowest 53 per cent, and the average 66.2 per cent; for yellow-green light (580 mμ) the highest was 92.5 per cent, the lowest 76.5 per cent, and the average 77.2 per cent; for red light (680 mμ) the lighest was 96 per cent, the lowest 73.5 per cent, and the average 82.7 per cent. Thus it is evident that in honeys which appear similar to the eye, there are considerable differences in the actual transmission of light of different wave lengths. Differences of even greater magnitude are found among darker honeys that are similar in appearance.

The data for honeys that were reported to have come from white clover (*Trifolium repens*) and alsike clover (*T. hybridum*) are shown in Table 3. The numbers in column 1 are those given the samples on arrival in the laboratory and are without significance as to grading.

TABLE 3.—*Percentages of light of different wave lengths passing through the honeys derived from white and alsike clovers*

Grade and honey No.	Blue light (480 mμ)	Yellow-green light (580 mμ)	Red light (680 mμ)	$\frac{R^a}{B}$	Grade and honey No.	Blue light (480 mμ)	Yellow-green light (580 mμ)	Red light (680 mμ)	$\frac{R^a}{B}$
Water White:	*Per ct.*	*Per ct.*	*Per ct.*		Light Amber:	*Per ct.*	*Per ct.*	*Per ct.*	
93	61.0	77.0	75.0	1.23	201	54.0	83.0	90.5	1.68
Extra White:					56	48.0	70.0	75.0	1.56
307	54.0	69.0	74.0	1.37	265	48.0	71.0	80.0	1.67
90	75.0	90.0	95.0	1.27	274	33.0	54.0	65.0	1.97
191	70.0	86.0	93.0	1.33	273	25.0	45.0	58.0	2.32
135	58.5	73.0	79.0	1.35	112	36.5	56.5	64.5	1.77
White:					302	41.5	69.0	80.0	1.93
193	61.5	79.0	89.0	1.45	177	47.5	78.5	83.0	1.75
219	59.0	77.0	80.0	1.36	31	37.5	64.0	68.5	1.83
256	58.0	74.0	79.0	1.36	154	30.5	51.0	58.0	1.90
95	45.0	59.5	63.0	1.40	23	44.0	68.0	74.0	1.68
314	50.0	73.0	77.0	1.54	60	41.0	70.0	77.5	1.89
200	56.0	77.5	84.0	1.50	271	32.5	62.5	71.0	2.18
272	50.0	73.0	80.0	1.60	Extra Amber:				
152	49.5	70.0	75.0	1.52	45	31.0	61.0	67.0	2.16
4	58.0	77.0	80.5	1.39	68	25.0	61.0	74.0	2.96
27	55.5	76.0	80.0	1.44	96	25.0	52.0	63.5	2.54
61	56.0	76.0	80.0	1.43	216	19.5	53.0	61.0	3.13
153	52.5	74.0	81.0	1.54	192	22.0	59.0	73.0	3.32
129	52.0	73.5	80.0	1.54	121	21.0	51.5	62.0	2.95
149	48.5	64.0	73.0	1.51	184	13.0	42.0	52.0	4.00
62	36.0	63.0	71.0	1.97	38	5.5	17.5	24.0	4.36
Extra Light Amber:					30	1.0	36.5	66.0	66.00
236	56.0	84.0	93.0	1.66	Maximum	75.0	90.0	95.0	1.27
91	56.0	80.0	89.0	1.59	Minimum	1.0	17.5	24.0	24.00
79	56.5	78.0	84.5	1.50	Average	44.0	67.5	75.0	1.70
108	51.0	74.0	77.0	1.51					
110	54.0	81.5	84.0	1.56					
200	54.0	84.5	88.0	1.63					

[a] Ratio of transmission of red light to transmission of blue.

It is evident from Table 3, which shows but a small portion of the original tables, that the color gradations of these honeys, as determined by experienced handlers of honey, fail to correspond with the gradations in light transmission for any one of the three wave lengths measured. So great is the variation in light transmission in honeys of practically the same color that it is necessary to find some other way to indicate differences in color.

It may be assumed that the grading of the 290 samples meets the demands of the American market. If this assumption is correct, the light transmissions for the various primary and secondary grades in these samples may be accepted as characteristic of honeys of the respective grades. Table 4 gives the data in condensed form for all of the 290 samples.

TABLE 4.—*Condensed data on light transmissions of the selected wave lengths for 290 honeys*

Grade	Samples	Blue light transmitted			Yellow-green light transmitted			Red light transmitted			$\frac{R^a}{B}$ of individual samples		
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Water White	24	80	53.0	66.2	92.5	67.5	77.2	96.0	73.5	82.5	1.43	1.15	1.25
Extra White	22	75	47.0	60.7	91	56.5	75.2	97.0	58.0	80.2	1.45	1.23	1.32
White	49	63	24.5	51.4	86.5	35.5	70.1	90.5	39.0	75.9	1.97	1.31	1.47
Extra Light Amber	13	56.5	40.0	50.4	84.5	62.5	74.4	93.0	70.0	80.9	1.85	1.48	1.61
Light Amber	68	54	23.5	39.1	86	41.0	65.5	90.5	50.5	73.9	2.43	1.56	1.90
Amber	84	41	4.0	21.3	82	17.5	55.4	92.0	24.0	66.8	14.75	1.86	3.62
Dark	30	22	0	3.25	58	.5	23.1	78.0	12.0	42.1	(b)	2.45	------

[a] Ratio of transmission of red light to transmission of blue. [b] Infinity.

THE POSSIBLE EFFECT OF RELATIVE SENSITIVITY OF THE EYE TO THE PROBLEM

The human eye does not perceive lights of all wave lengths equally. Table 5, which is taken from the work of Coblentz and Emerson,[9] shows the relative sensitivity of the eye to various colors. It is based on observations with 130 individuals made by detecting the disappearance of flicker when two lights of different colors and intensities were alternated rapidly.

TABLE 5.—*Relative sensitivity of the eye to lights of various colors* [a]

Wave length (mμ)	Visibility	Wave length (mμ)	Visibility	Wave length (mμ)	Visibility	Wave length (mμ)	Visibility
440	0.033	520	0.710	600	0.687	660	0.0645
460	.056	540	.954	620	.427	680	.0178
480	.125	560	.998	640	.194	700	.0040
500	.316	580	.898				

[a] After Coblentz and Emerson. Op. cit., Table 5, p. 219.

It is evident from Table 5 that the human eye perceives light most acutely in the region of 560 mμ (yellow-green) and much less acutely at both the violet (440 mμ) and red (700 mμ) ends of the visible spectrum.

It might be suspected that the relative sensitivity of the eye to lights of various colors has some effect on the color impressions arising from the passage of light through honeys. This seems not to be the case, however, for the relative sensitivity is the same for the light either before or after it passes through the honey. No results of value have been obtained from attempts to calculate the light transmission of the various honeys in relation to the relative sensitivity of the eye to colors, since nothing approaching regularity has been found in such computations.

THE RATIO OF TRANSMISSIONS AS A BASIS FOR GRADING

As previously stated, differential absorption of the white light gives the impression of colors that we get from looking at honeys. If the light which passes through honey has exactly the same relative proportions of the various wave-length components as are found in the original white light which strikes the honey, our impression would be that the honey is white. If some light is absorbed more than others, we get different color impressions, depending on which wave lengths of light pass without absorption.

Before discussing this subject in detail, reference may be made to the "color" often seen in honey from fireweed or willow herb (*Epilobium angustifolium*). This honey would usually be graded as Water White or Extra White, fireweed honey being one of the lightest to be found anywhere. A pure fireweed honey is so nearly devoid of amber

[9] COBLENTZ, W. W., and EMERSON, W. B. RELATIVE SENSIBILITY OF THE AVERAGE EYE TO LIGHT OF DIFFERENT COLORS AND SOME PRACTICAL APPLICATIONS TO RADIATION PROBLEMS. U. S. Dept. Com., Bur. Standards Bul. 14: 167–236, illus., 1918.

color that it often has a slightly gray appearance. This appearance of grayness arises from the fact that all wave lengths of light are absorbed in almost the same proportions, and the light which passes through a stratum of this honey is therefore practically white light. There is, however, some absorption of lights of all wave lengths, so that the amount of light which passes through the honey is less than that which comes around a jar. This reduction in the light as it passes through the honey gives merely an impression of slight grayness, without any noticeable color. Even for fireweed honey there is always slightly greater absorption of blue than of red light, as indicated in Table 2.

It is, then, possible for a honey to give the impression of being white or slightly gray even though a considerable proportion of the light which strikes it is absorbed. Although entirely equal absorption of all wave lengths has not been found for any honey, the human eye gives a color value, however slight, to all honeys. Usually this color is described as some shade of amber, the implication being that the wave lengths of light toward the red end of the visible spectrum pass through the honey more readily than do those of the violet end. An examination of the data for the clover honeys (Table 3) shows that one honey allowed 95 per cent of the red light (680 mμ) to pass through a stratum of 1 cm, whereas the greatest transmission of blue light (480 mμ) was 75 per cent. The averages for the clover honeys were as follows: Blue (480 mμ), 44 per cent; yellow-green (580 mμ), 67.5 per cent; red (680 mμ), 75 per cent. These figures support the previous statement that honeys permit more red light to be transmitted than any other, the violet end of the visible spectrum being most heavily absorbed.

It is possible for two honeys to have the same ratio of light transmission in the several wave lengths and still to absorb quite different percentages of the light of each of the three wave lengths. In such an event the honeys would have the same color, yet one would actually transmit less light than the other. As one goes toward the darker grades of honey, one finds that more and more light is absorbed but that there is a most rapid reduction in the transmission of light toward the violet end of the spectrum. It therefore seems at least theoretically possible to use as a factor in establishing the color grades of honey the ratio obtained by dividing the percentage transmission of red light by the percentage transmission of blue light. This ratio would be a constantly increasing number from the lightest to the darkest honeys.

The clover honeys have been examined on this basis. The lowest ratio found is 1.23 for the lightest honey, and the highest ratio is 66 for the darkest. (Table 3.) There is a steady increase in the ratio from the lightest to the darkest clover honey, but the rate of increase is not entirely uniform because of the smallness of the series. There are also certain variations which can not readily be explained on the basis of measurements with the spectrophotometer alone.

Since the actual figures representing the percentages of light of various wave lengths fail to give any satisfactory means of delimiting the color grades, and since the ratio of red to blue gives a more satis-

factory way of indicating these limits, it seems possible that the definitions of the honey color grades might be based chiefly on this ratio. The figures published by Sechrist and Samson [10] seem to be of some service, however, for while they can not be accepted as the lower limits of light transmissions for the several grades, they may still be useful as indicators of the percentage of light absorption to be expected toward the lower limits of the grades. It therefore seems wise not to abandon these figures entirely but to use them as examples. They will serve as a further check, in the event that later attempts are made to devise practical grading instruments for the use of beekeepers and honey dealers.

It may be admitted that no optical physicist would probably ever think of using such a ratio of light transmissions as is here suggested. However, since the ratio seems to serve as a useful method for defining the honey color grades, being far superior to any other attempt made to define them, there seems to be no valid reason for refusing to accept it merely because the method is unusual.

The reduction in light transmission is seen to be regularly such that the red exceeds the yellow-green and the yellow-green the blue. On the basis of the suggestion made earlier, it might then be possible to use the factor $\frac{R}{B}$, $\frac{R}{Y}$, or $\frac{Y}{B}$. These factors appear to be equally valid, but of course the one should be chosen that gives the most consistent and definite results. As might be expected, the extremes best meet this requirement.

It would also be possible to get still larger numbers for the ratio if wave lengths other than 480 mμ and 680 mμ were chosen. For example, if 440 mμ and 700 mμ were used, the ratio would be larger, but in that event the wave lengths measured would approach the limits of human vision, which would make the measurements less definite. Since the selection of any two measurements is wholly arbitrary, it is necessary only to get data on wave lengths far enough apart to show significant ratios.

By computing the three factors mentioned for all the honeys in averages for groups of tens, all light transmissions for each group being averaged, the usefulness of these three factors has been tested. For $\frac{Y}{B}$ there are five instances in which a group of 10 honeys give a ratio on this basis which is lower than that of the group of 15 honeys immediately adjacent on the light side. For $\frac{R}{Y}$, there are 10 such exceptions. For $\frac{R}{B}$ there are found only 3, perhaps as good a showing as can be found for any grading scheme for honeys. These ratios are given in Table 6.

[10] SECHRIST, E. L., and SAMSON, H. W. Op. cit.

TABLE 6.—*Average percentage light transmission for the lightest 260 honeys in groups of 10 each, with computed ratios as indicated*

[The 30 darkest honeys are omitted]

Group No.	Percentage transmission of—			$\frac{R}{B}$	$\frac{R}{Y}$	$\frac{Y}{B}$
	Blue light	Yellow-green light	Red light			
1	70.3	80.1	86.0	1.23	1.08	1.14
2	62.7	74.2	78.9	1.26	[a] 1.06	1.19
3	63.4	76.8	82.2	1.30	1.07	1.21
4	58.1	71.7	76.7	1.32	1.07	1.24
5	62.4	79.2	84.1	1.35	[a] 1.06	1.27
6	58.7	75.1	81.5	1.39	1.10	1.29
7	50.8	69.0	74.2	1.46	[a] 1.08	1.36
8	54.4	75.1	80.9	1.49	[a] 1.08	1.38
9	45.5	64.7	70.5	1.55	[a] 1.09	1.42
10	42.7	64.6	73.0	1.72	1.13	1.52
11	51.2	75.6	80.6	[a] 1.58	[a] 1.07	[a] 1.48
12	43.8	66.6	75.0	1.73	1.13	1.53
13	40.6	66.3	75.0	1.88	1.14	1.64
14	41.4	67.2	74.8	[a] 1.81	[a] 1.12	[a] 1.62
15	38.4	67.1	74.1	1.94	[a] 1.11	1.75
16	37.7	64.2	74.2	1.99	1.16	[a] 1.72
17	34.0	62.0	69.9	2.56	[a] 1.13	1.83
18	32.6	63.0	71.7	2.20	[a] 1.14	1.94
19	29.3	61.5	70.1	2.44	[a] 1.15	2.13
20	25.0	58.0	68.5	2.84	1.19	2.39
21	24.8	55.6	65.6	[a] 2.67	[a] 1.18	[a] 2.27
22	22.6	56.0	71.6	3.33	1.28	2.74
23	21.7	55.7	69.2	3.48	[a] 1.25	[a] 2.66
24	17.4	54.6	66.6	4.09	[a] 1.23	3.35
25	14.5	47.0	60.5	4.34	1.30	3.35
26	11.4	47.0	60.4	6.32	[a] 1.29	4.85

[a] These ratios show irregularities in the progression from the lighter to the darker honeys.

If this proposed grading plan is subjected to still another test an examination of the ratios $\frac{R}{B}$ for the individual honeys of the entire series only six cases will be found of honeys which have a ratio higher than the average ratio of the next lower grade. These six exceptions should be contrasted with the 263 exceptions found on examination of the definitions of grades formerly published. Three of these exceptions occur in the Water White grade and three in the White grade. None appear in the darker grades, and in both instances the exceptions occur because of the interpolation of secondary (extra) grades.

CONCLUSIONS

As a result of a study of the transmission of light of three wave lengths through 290 honeys which had been arranged in series and marked as to their proper market grade by expert honey graders, the definitions for honey color grades shown in Table 7 are proposed.

TABLE 7.—*Proposed definitions of honey color grades based on transmission of blue, yellow-green, and red light and on the ratios between the transmissions of the red and blue lights*

Grade	Approximations of light transmission for the lower limits of the grades			Ratio of red light transmission to blue $\left(\frac{R}{B}\right)$	
	Blue light (480 mμ)	Yellow-green light (580 mμ)	Red light (680 mμ)	Maximum	Average
	Per cent	*Per cent*	*Per cent*		
Water White	64.0	77.0	81.5	1.43	1.25
Extra White	60.0	76.0	80.5	1.45	1.32
White	50.0	71.0	78.0	1.61	1.47
Extra Light Amber	48.0	70.0	77.0	1.97	1.61
Light Amber	34.5	63.0	73.0	2.43	1.90
Extra Amber	29.0	60.5	70.5	[a] 4.20	([a])
Amber	15.0	49.0	63.0	8.00	3.62
Dark Amber	6.0	43.0	59.0	[a] 9.83	([a])
Dark [b]	([b])	([b])	([b])	([c])	?

[a] Averages are not given for the grades Extra Amber and Dark Amber, since no definite division points for these grades were designated in the 290 honeys studied. The maximum ratios are based on estimates of the probable maximum if the secondary grades are to include roughly 30 per cent of the primary grades.
[b] All honeys darker than Amber (or Dark Amber).
[c] Infinity.

The figures in Table 7 for maximum ratio of red light transmission to blue light transmission differ from those given in Table 4 for the same grades in three instances. These changes are proposed because the extreme figures for ratios in Table 4 were those of three honeys toward the lower end of the respective grades (White, Extra Light Amber, and Amber) which may as well be allowed to fall into darker grades. In no case did these exceptional honeys lie farther than two or three from the darker limits of the grades. These changes are proposed also because the ratios of the particular honeys which gave abnormally high ratios are considerably higher than those of honeys immediately adjacent to them in the series.

If color-grading rules based on these figures are adopted, it is believed that it will be possible for any properly equipped manufacturing concern to make a honey color grader that will meet the demands of the definitions. Moreover, the color grades will be accurately defined, which so far has not been the case.

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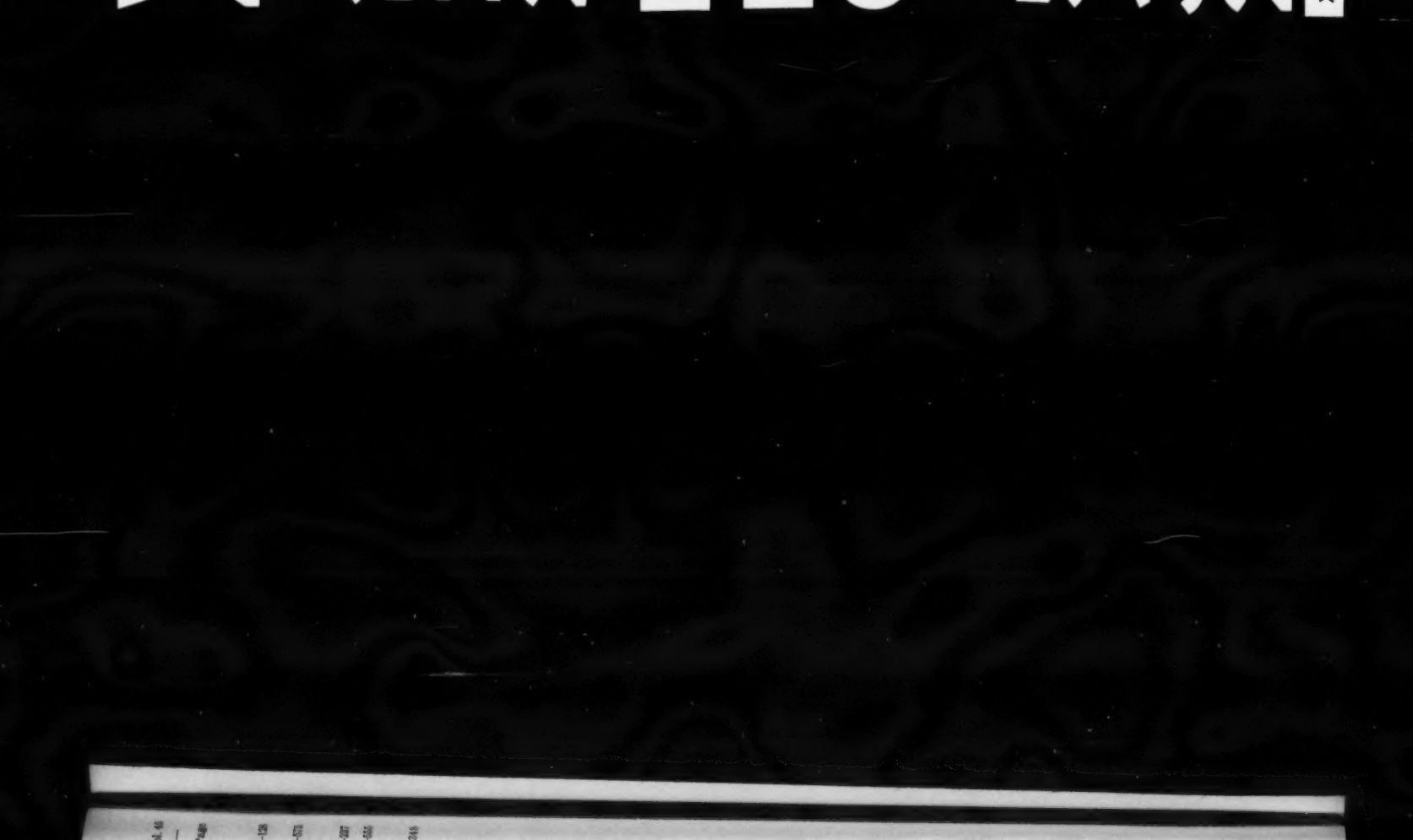
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